



US 20130344553A1

(19) **United States**

(12) **Patent Application Publication**
Lee

(10) **Pub. No.: US 2013/0344553 A1**

(43) **Pub. Date: Dec. 26, 2013**

(54) **DESIGNER CALVIN-CYCLE-CHANNELED AND HYDROGENOTROPHIC PRODUCTION OF BUTANOL AND RELATED HIGHER ALCOHOLS**

61/066,835, filed on Feb. 23, 2008, provisional application No. 61/426,147, filed on Dec. 22, 2010.

Publication Classification

(76) Inventor: **James Weifu Lee**, Cockeysville, MD (US)

(51) **Int. Cl.**
C12P 7/16 (2006.01)

(21) Appl. No.: **13/997,242**

(52) **U.S. Cl.**
CPC **C12P 7/16** (2013.01)
USPC **435/160; 435/157**

(22) PCT Filed: **Dec. 20, 2011**

(86) PCT No.: **PCT/US11/66090**

§ 371 (c)(1),
(2), (4) Date: **Jun. 22, 2013**

(57) **ABSTRACT**

Designer Calvin-cycle-channeled and hydrogenotrophic bio-fuel-production pathways, the associated designer genes and designer transgenic organisms for autotrophic production of butanol and related higher alcohols from carbon dioxide, hydrogen, and/or water are provided. The butanol and related higher alcohols include 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, and 6-methyl-1-heptanol. The designer autotrophic organisms such as designer transgenic oxyphotobacteria and algae comprise designer Calvin-cycle-channeled and hydrogenotrophic pathway gene(s) and biosafety-guarding technology for enhanced autotrophic production of butanol and related higher alcohols from carbon dioxide and water.

Related U.S. Application Data

(63) Continuation-in-part of application No. 13/075,153, filed on Mar. 29, 2011, which is a continuation-in-part of application No. 12/918,784, filed on Aug. 20, 2010, filed as application No. PCT/US2009/034801 on Feb. 21, 2009.

(60) Provisional application No. 61/426,147, filed on Dec. 22, 2010, provisional application No. 61/066,845, filed on Feb. 23, 2008, provisional application No.

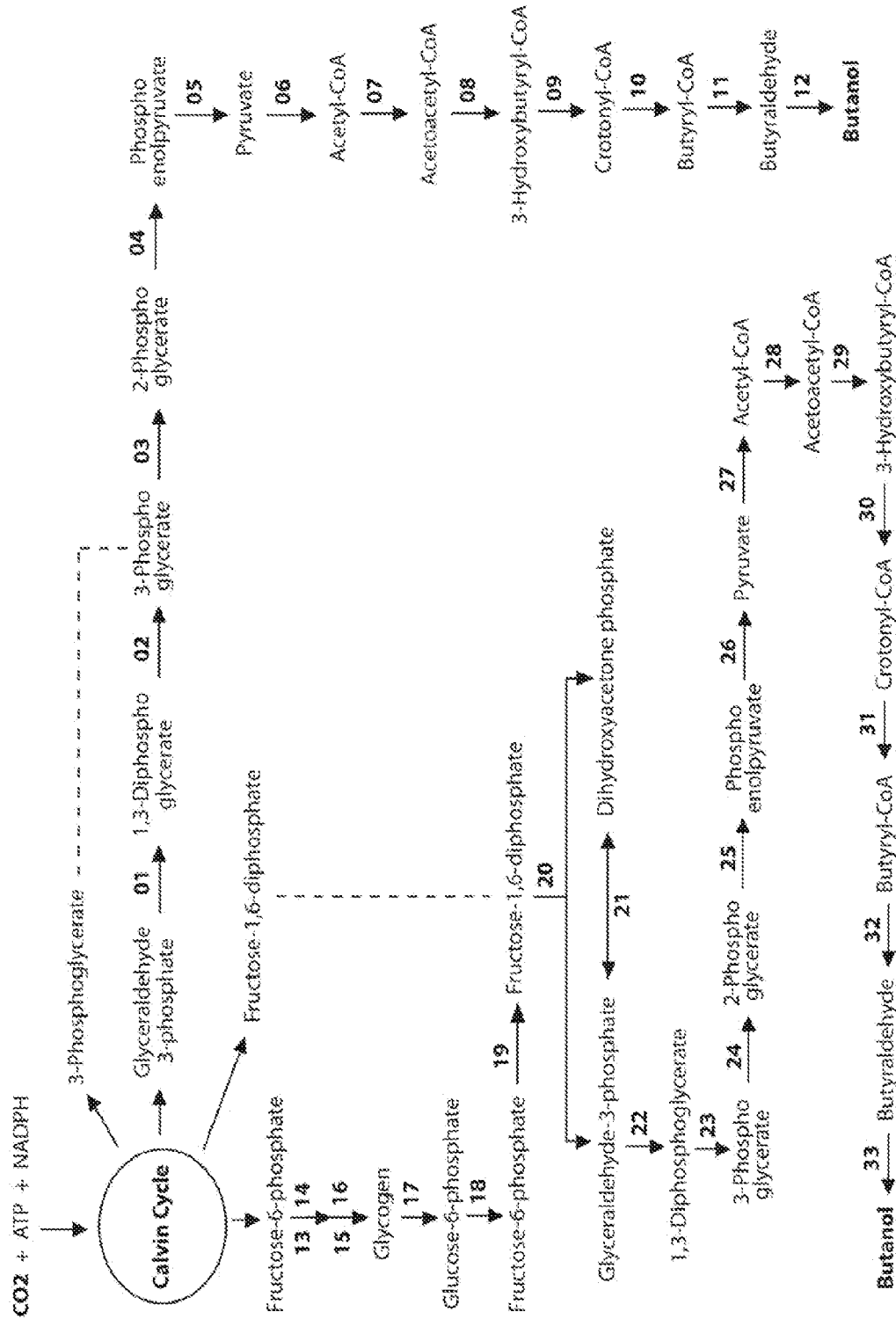


FIG. 1

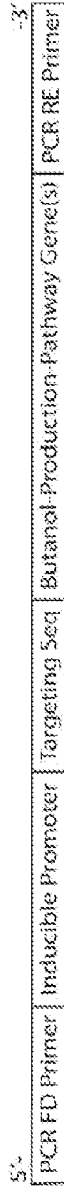


FIG. 2A

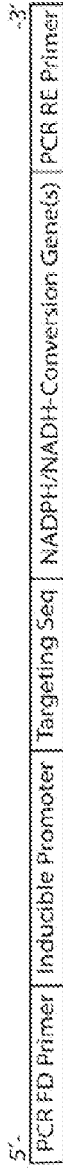


FIG. 2B



FIG. 2C

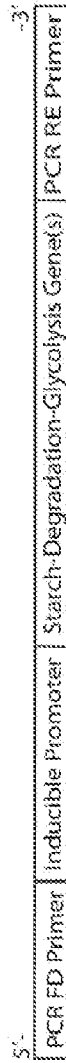


FIG. 2D

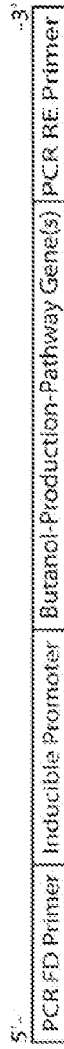


FIG. 2E

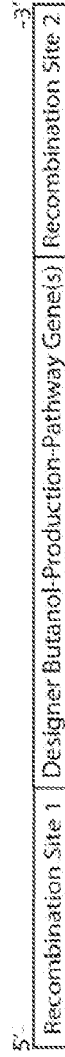


FIG. 2F

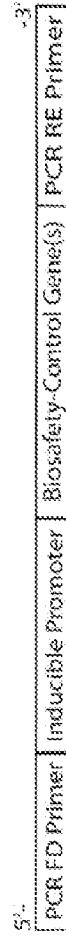


FIG. 2G

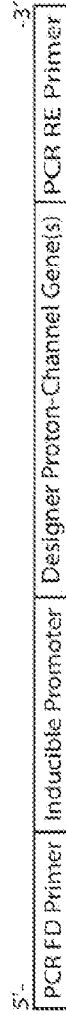


FIG. 2H

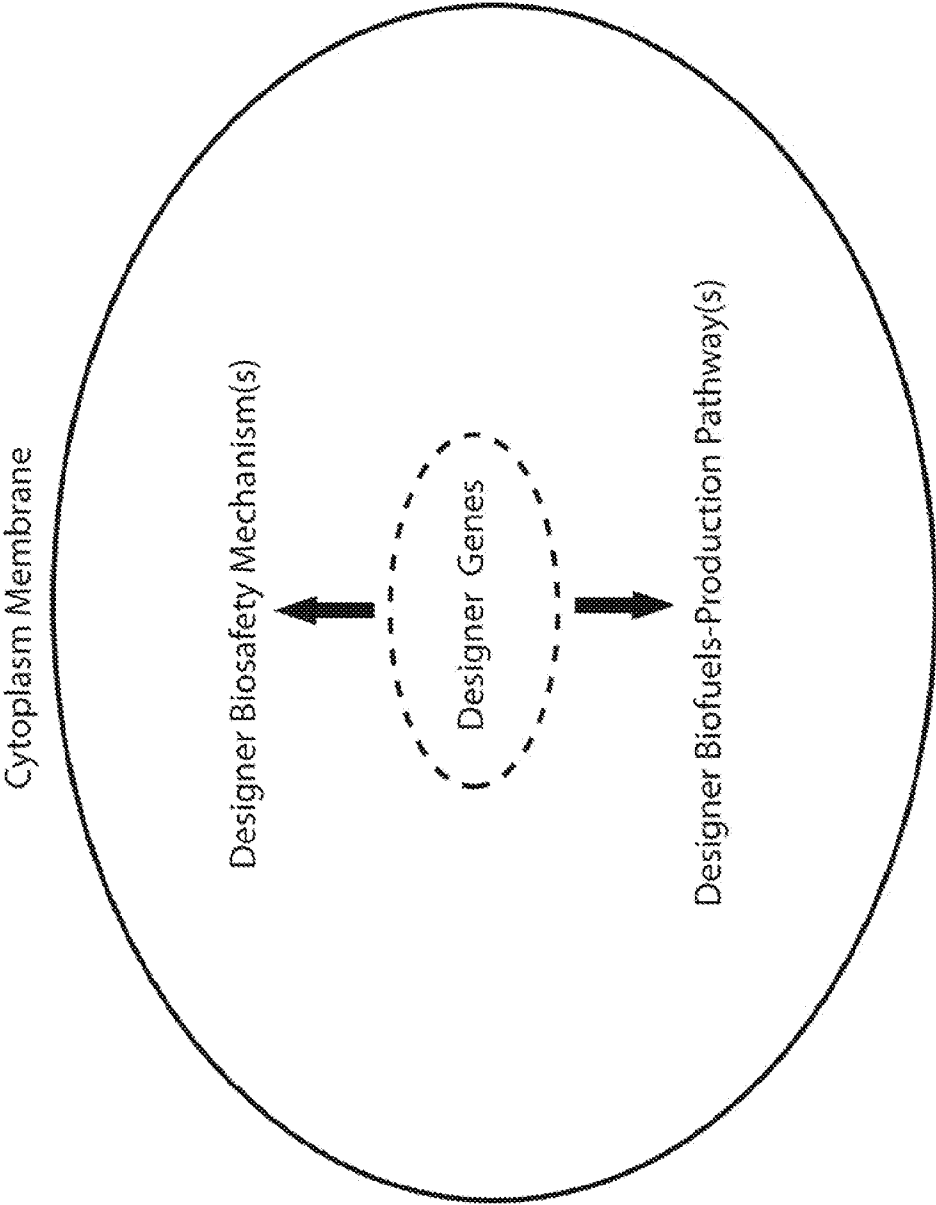


FIG. 3A

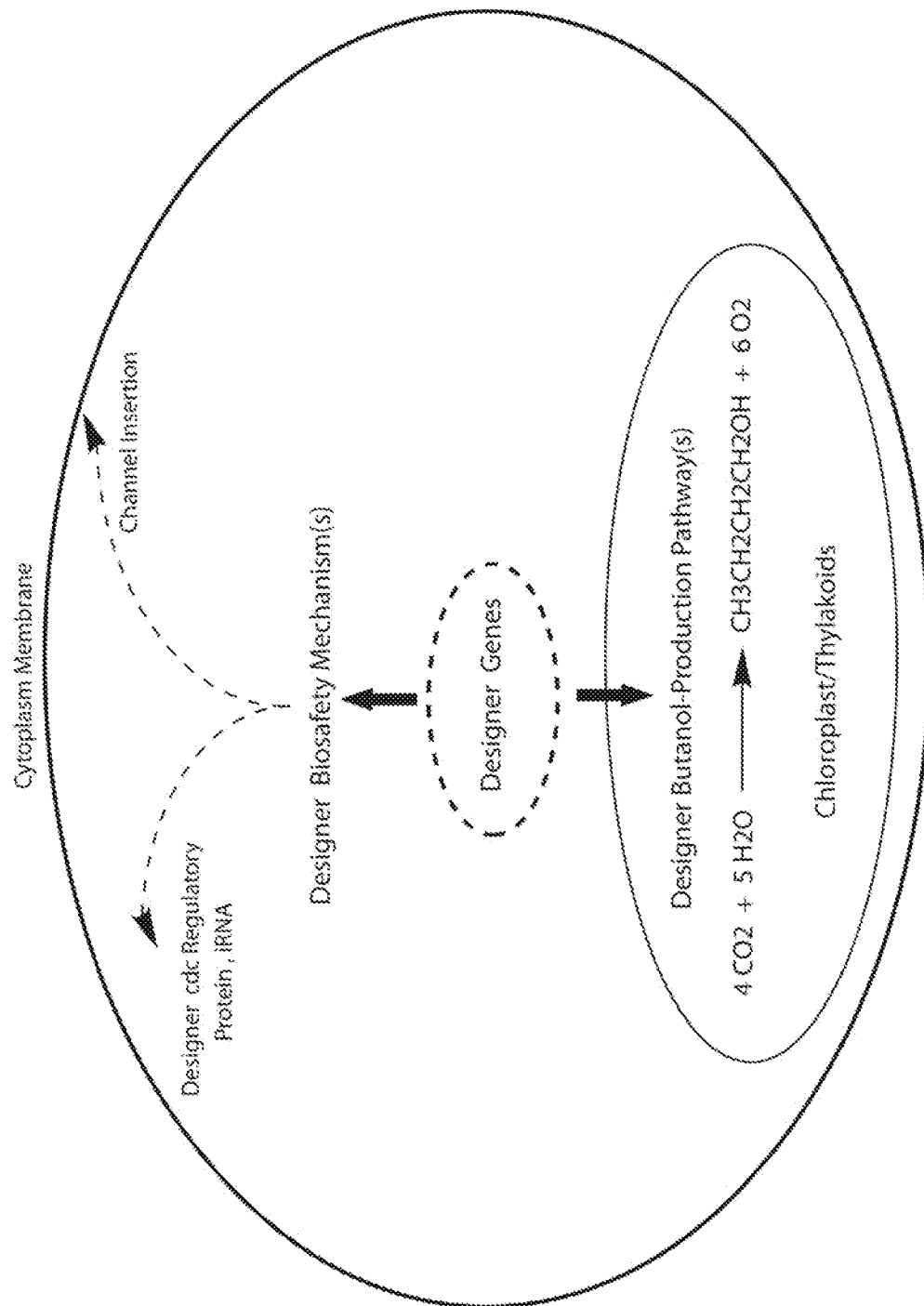


FIG. 3B

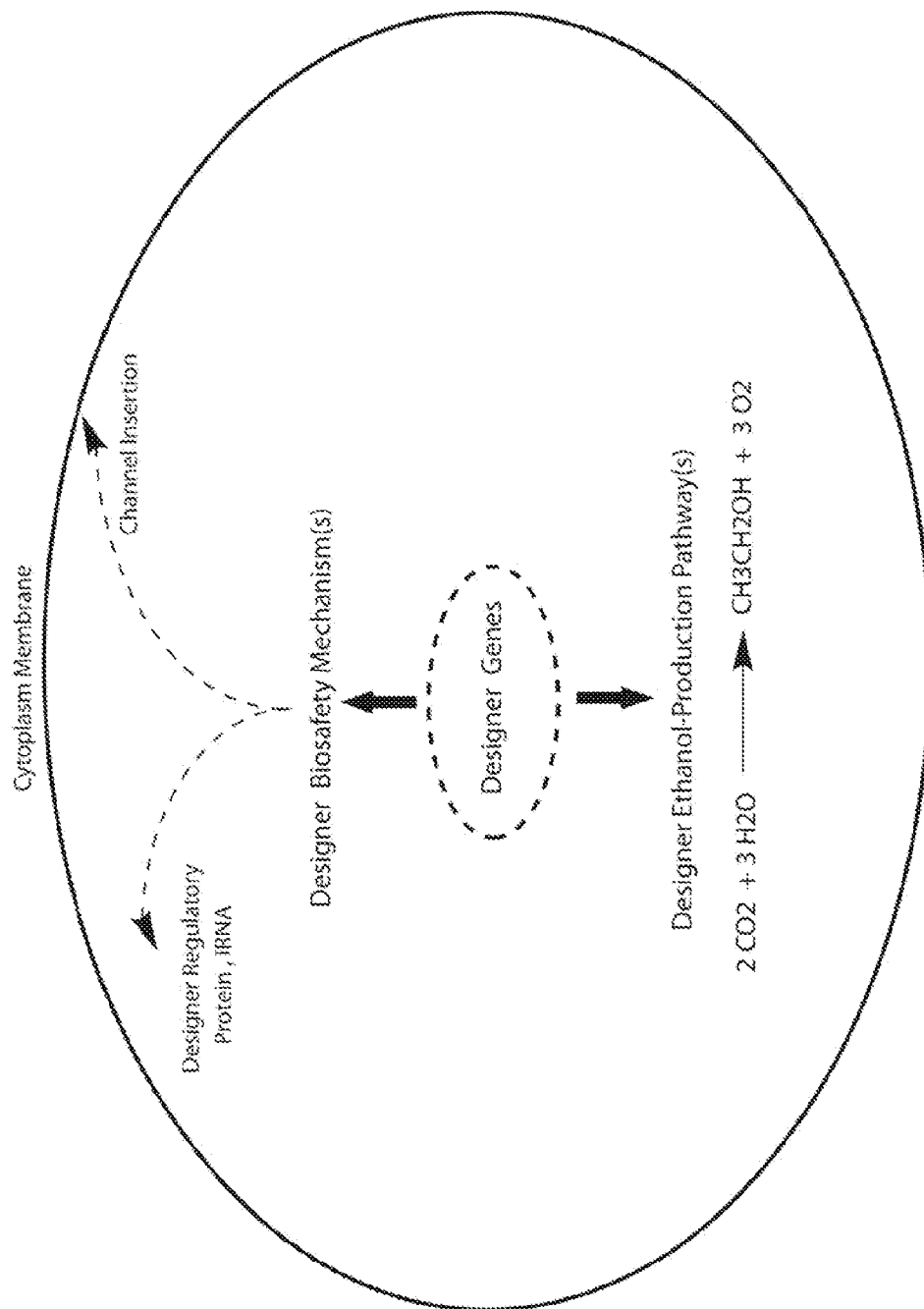


FIG. 3C

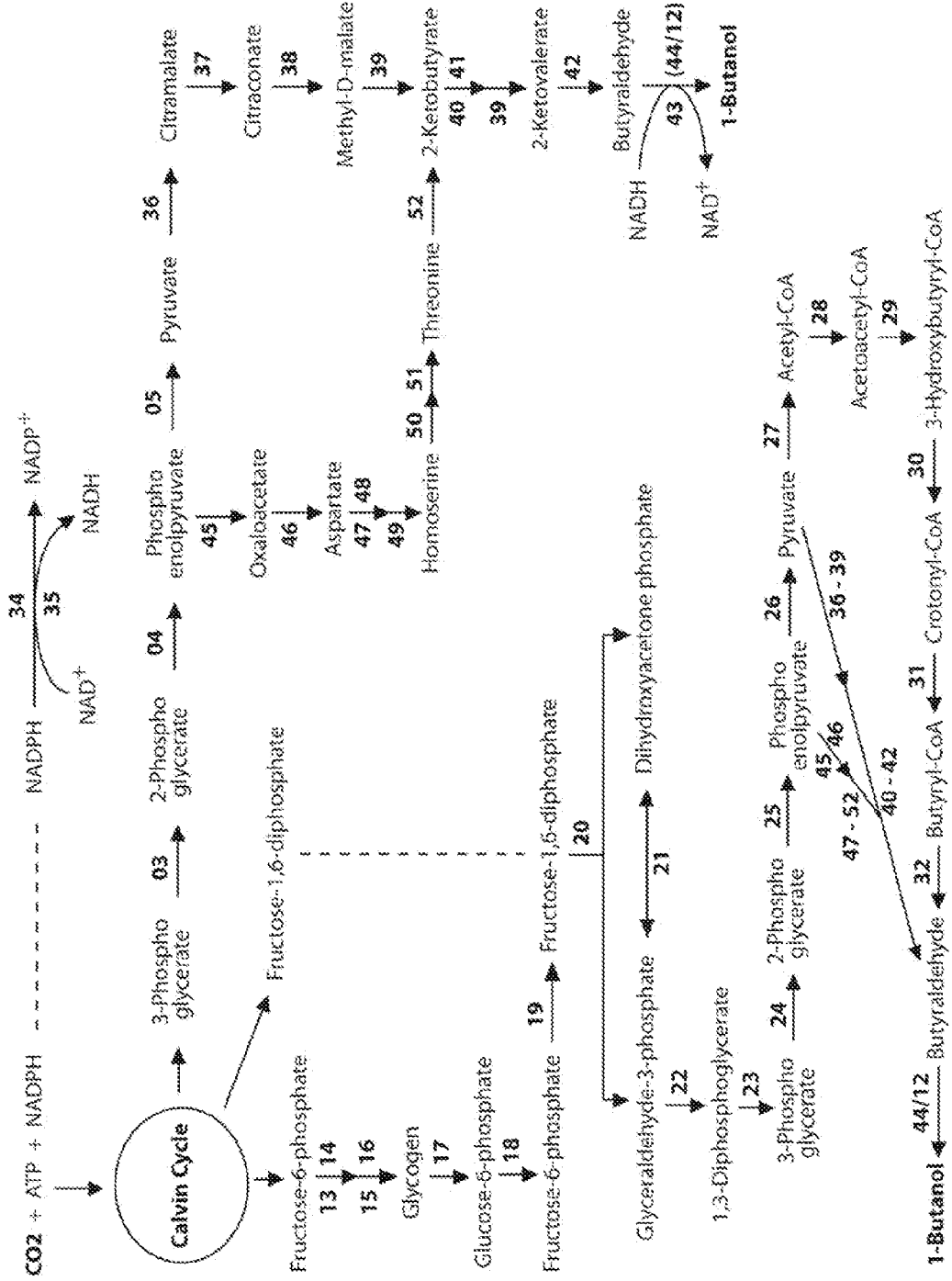


FIG. 4

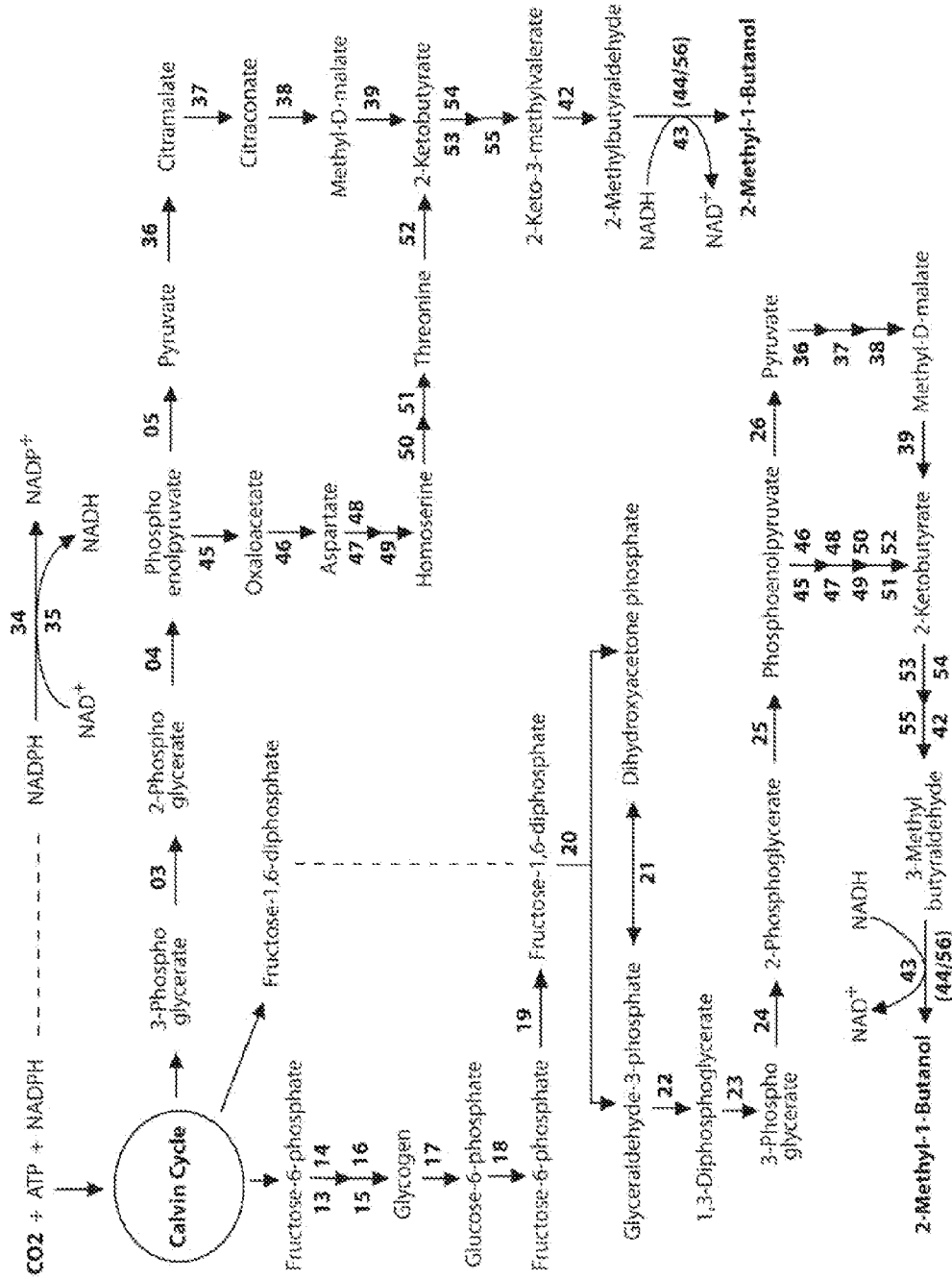


FIG. 5

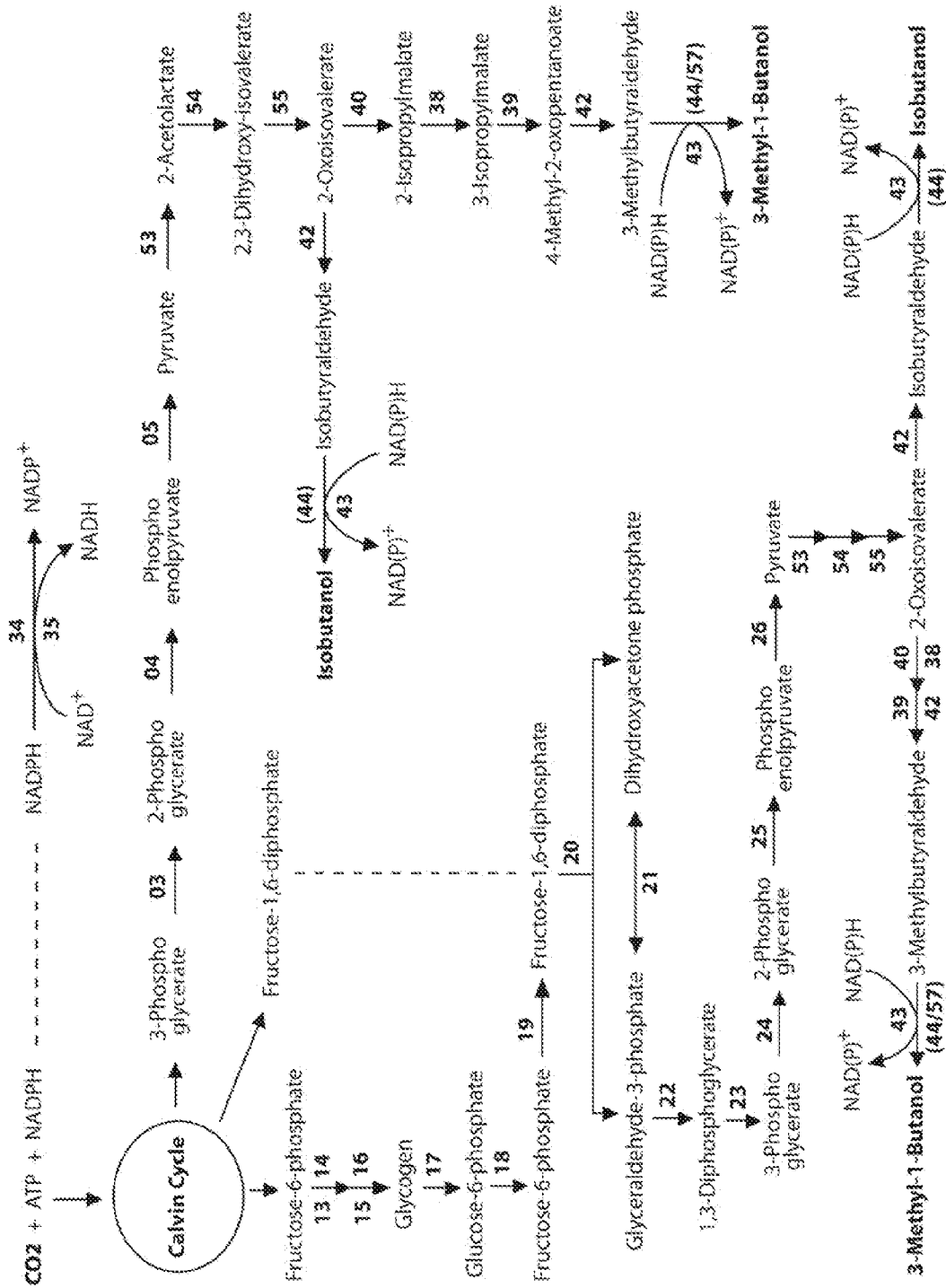


FIG. 6

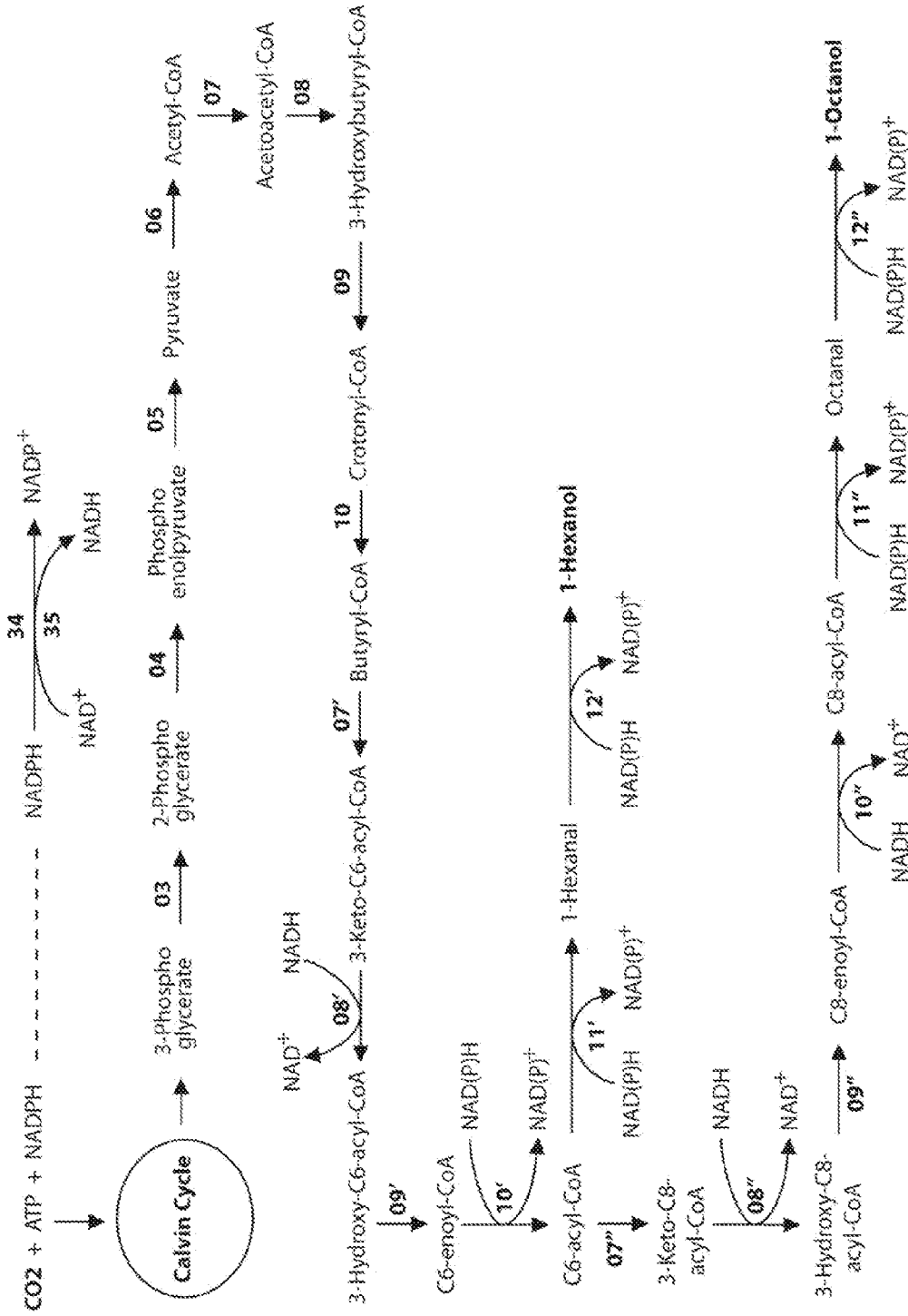


FIG. 7

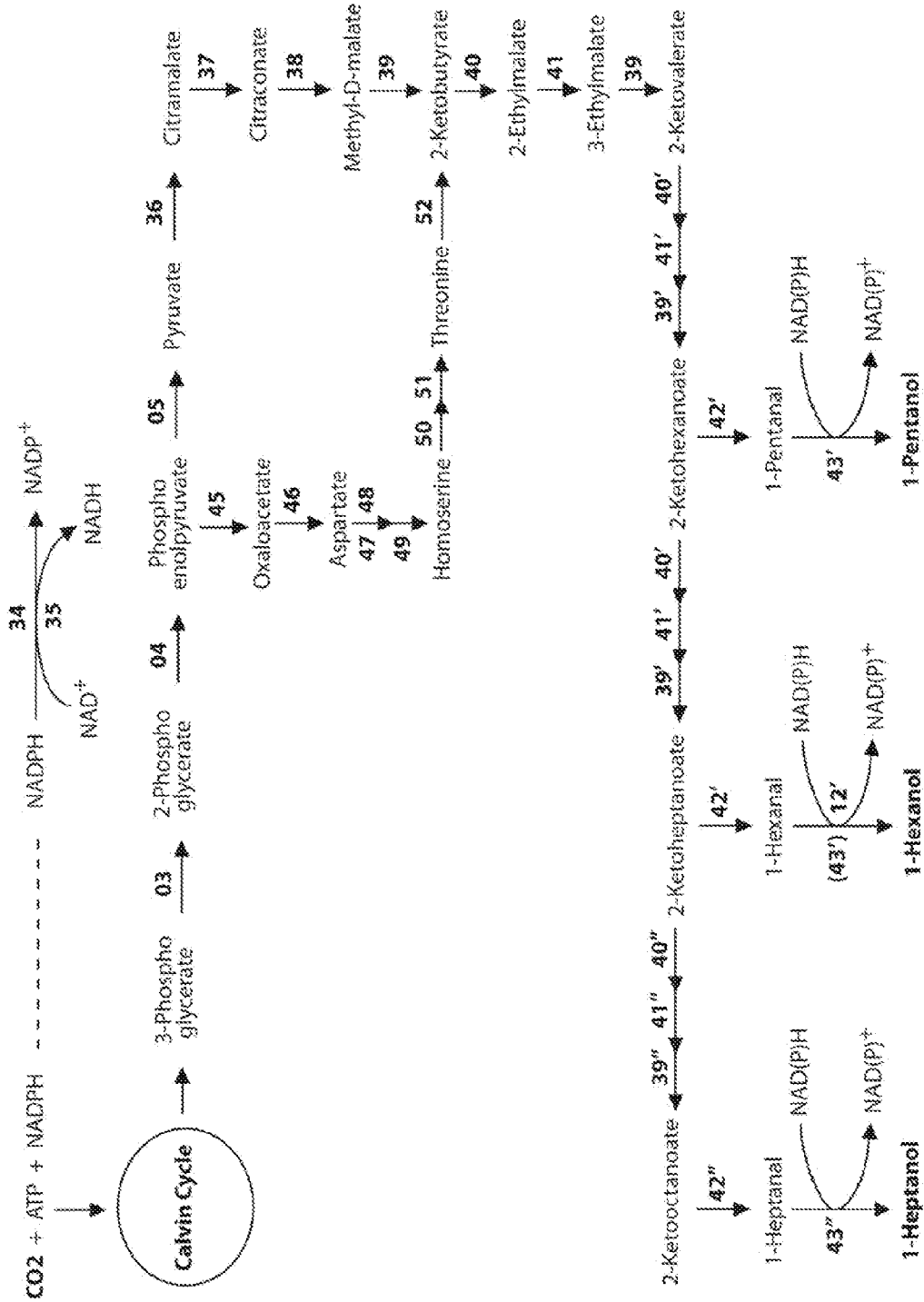


FIG. 8

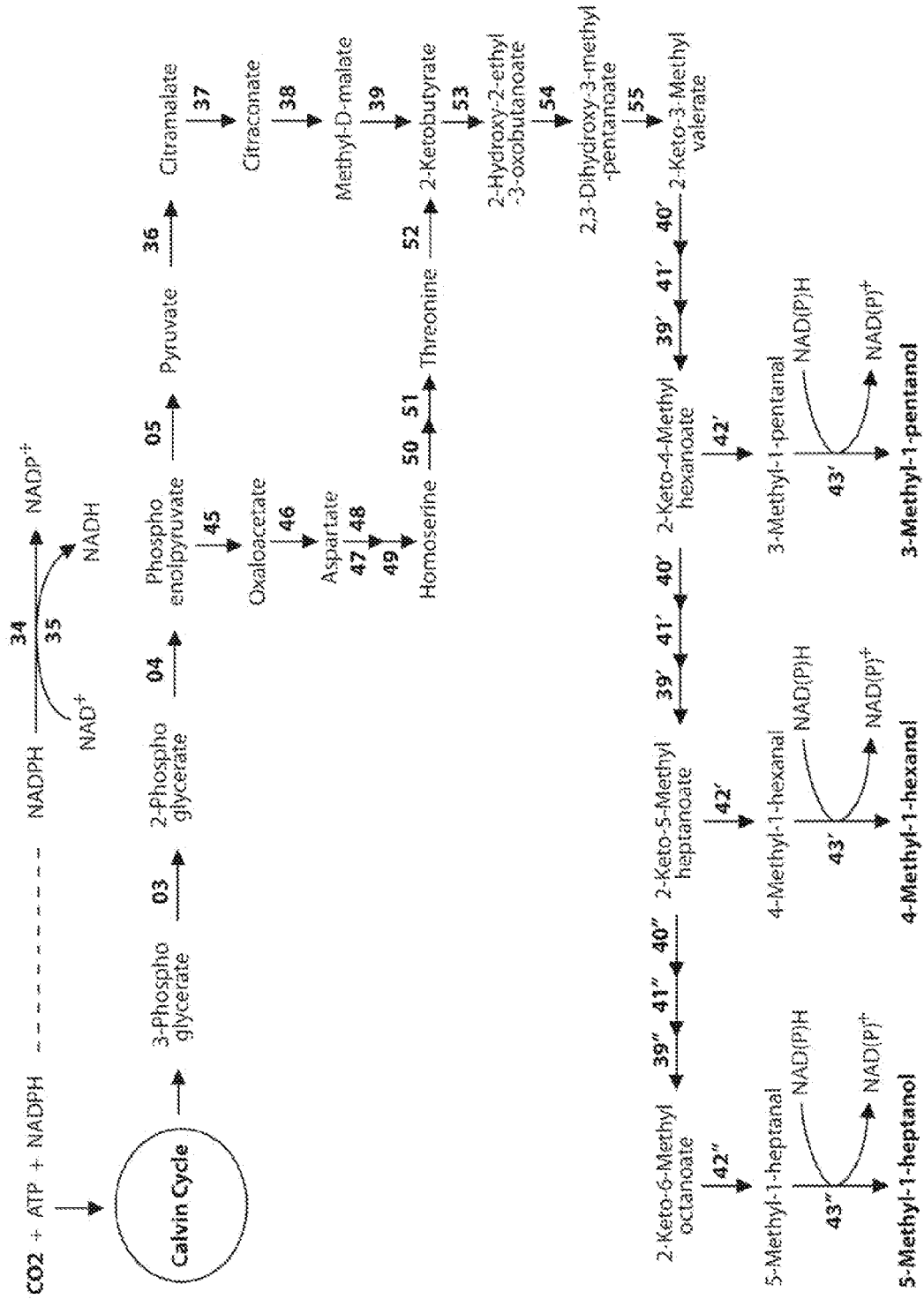


FIG. 9

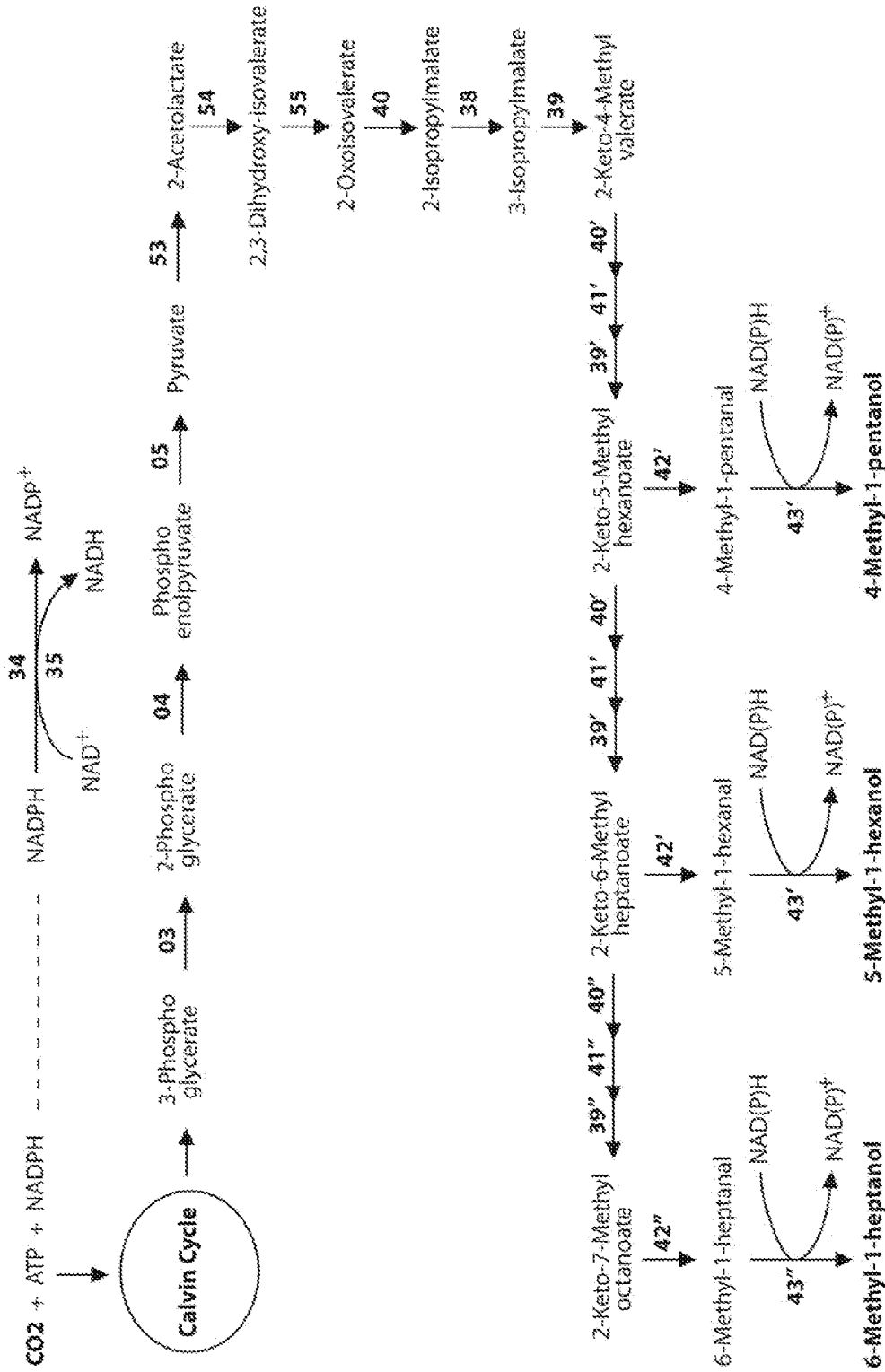


FIG. 10

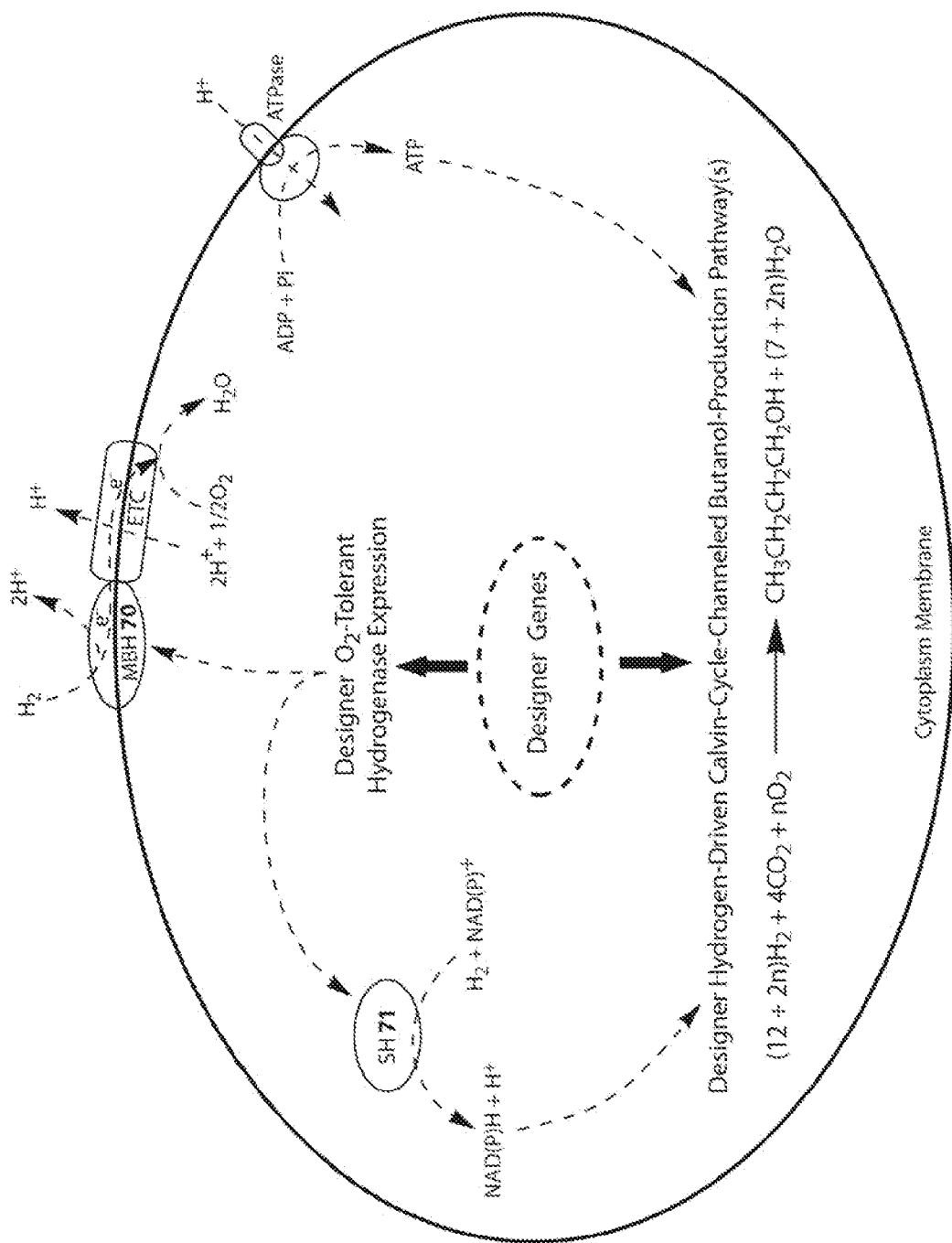


FIG. 11

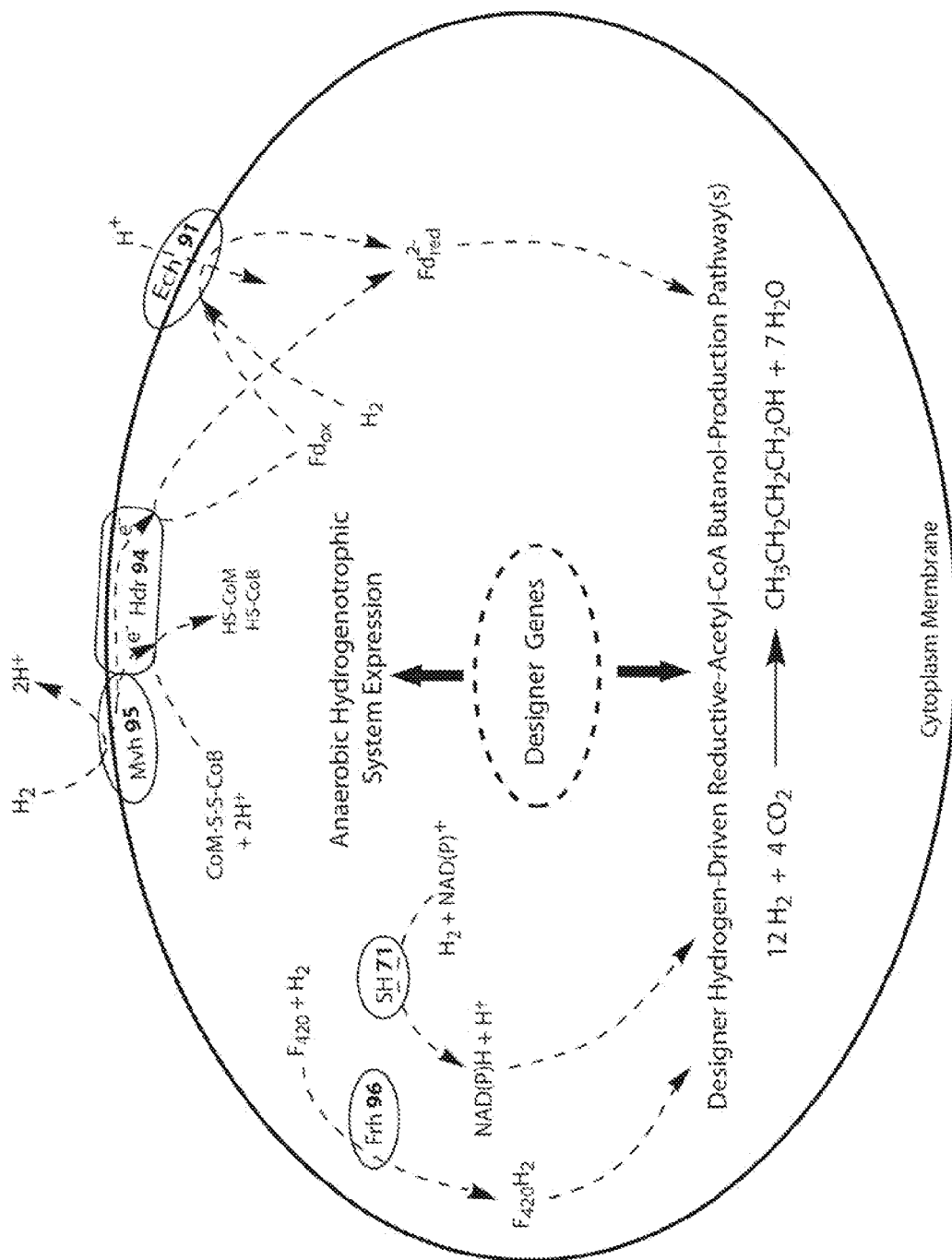


FIG. 12

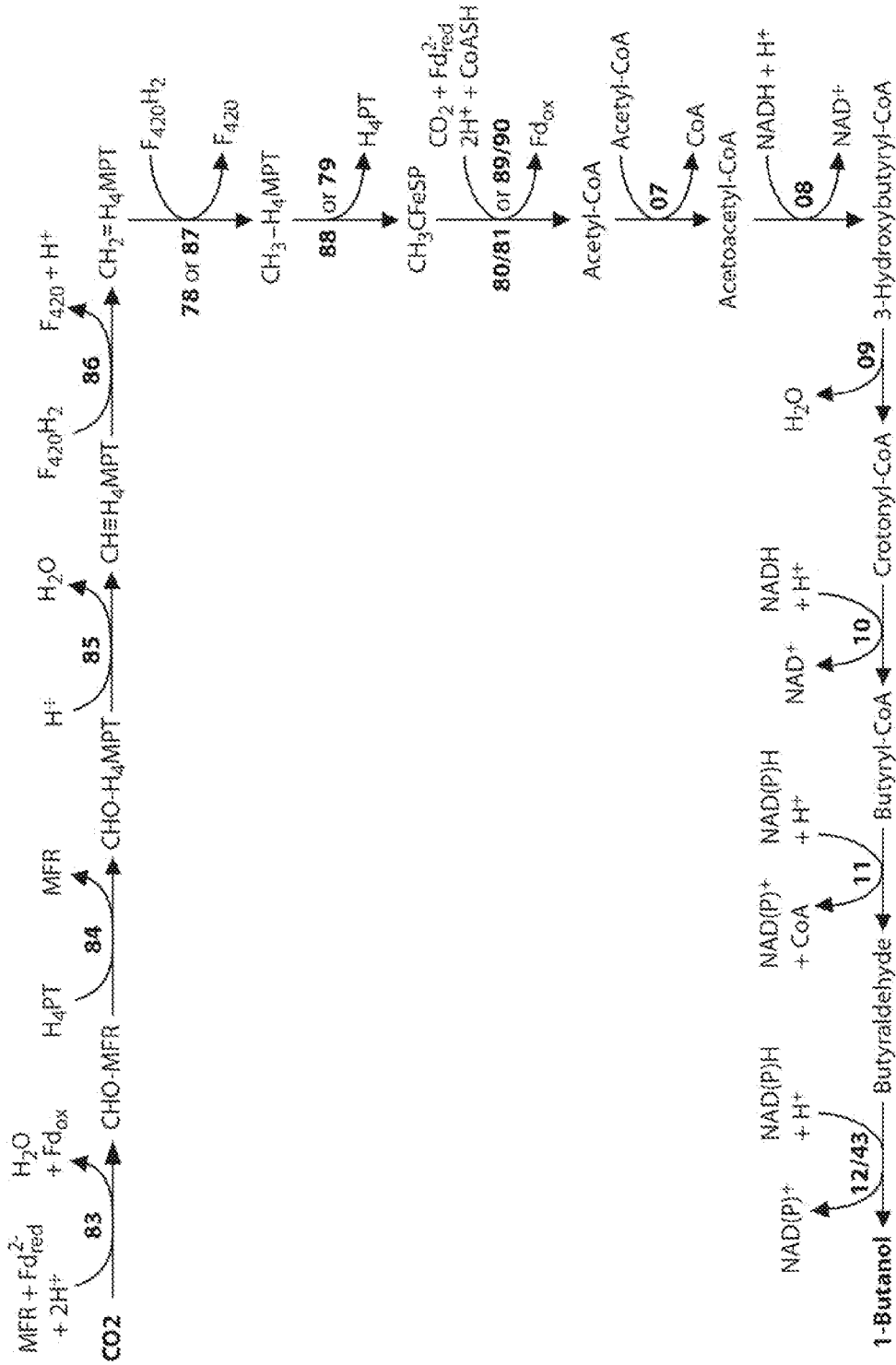


FIG. 13

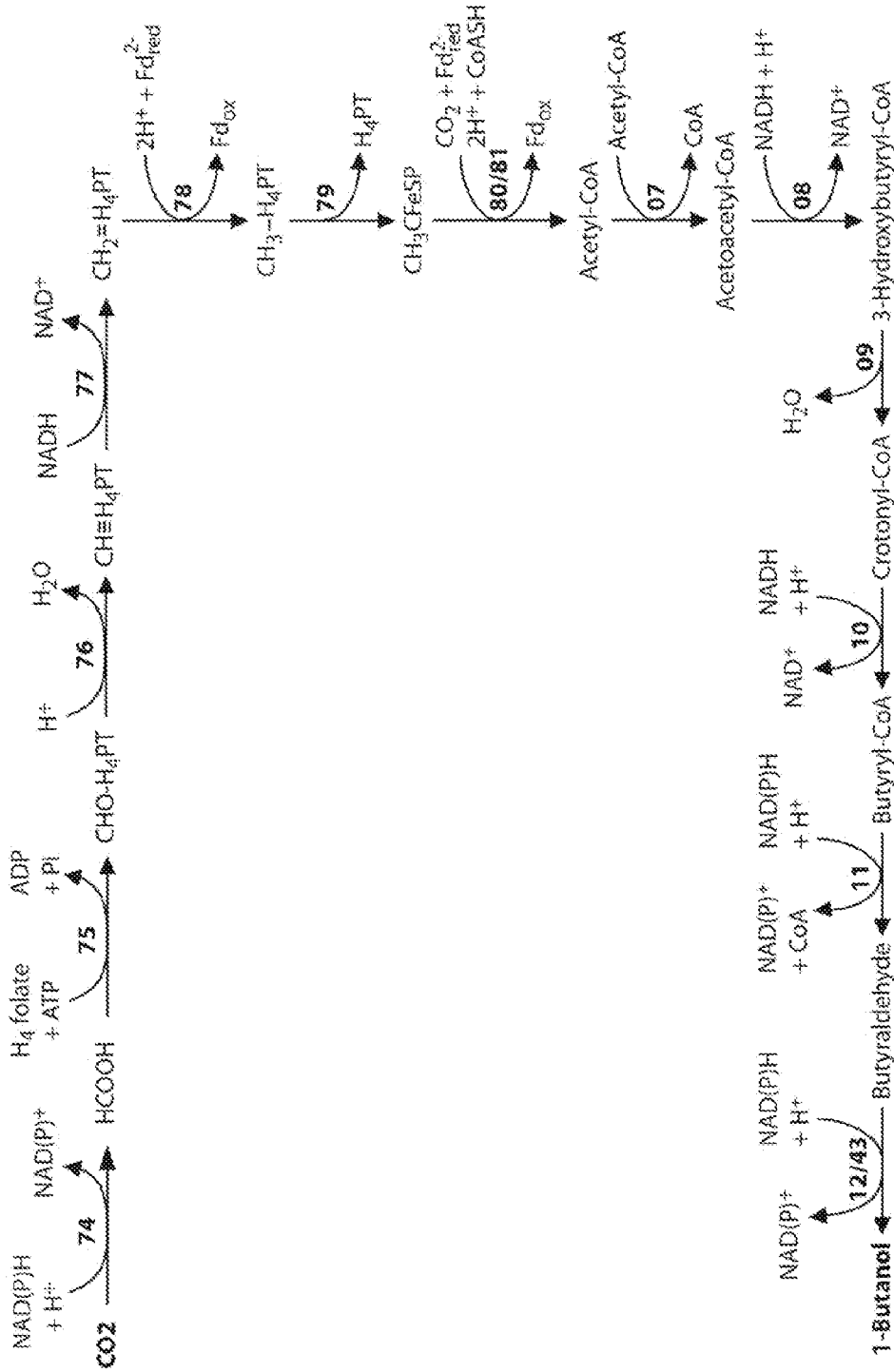


FIG. 14

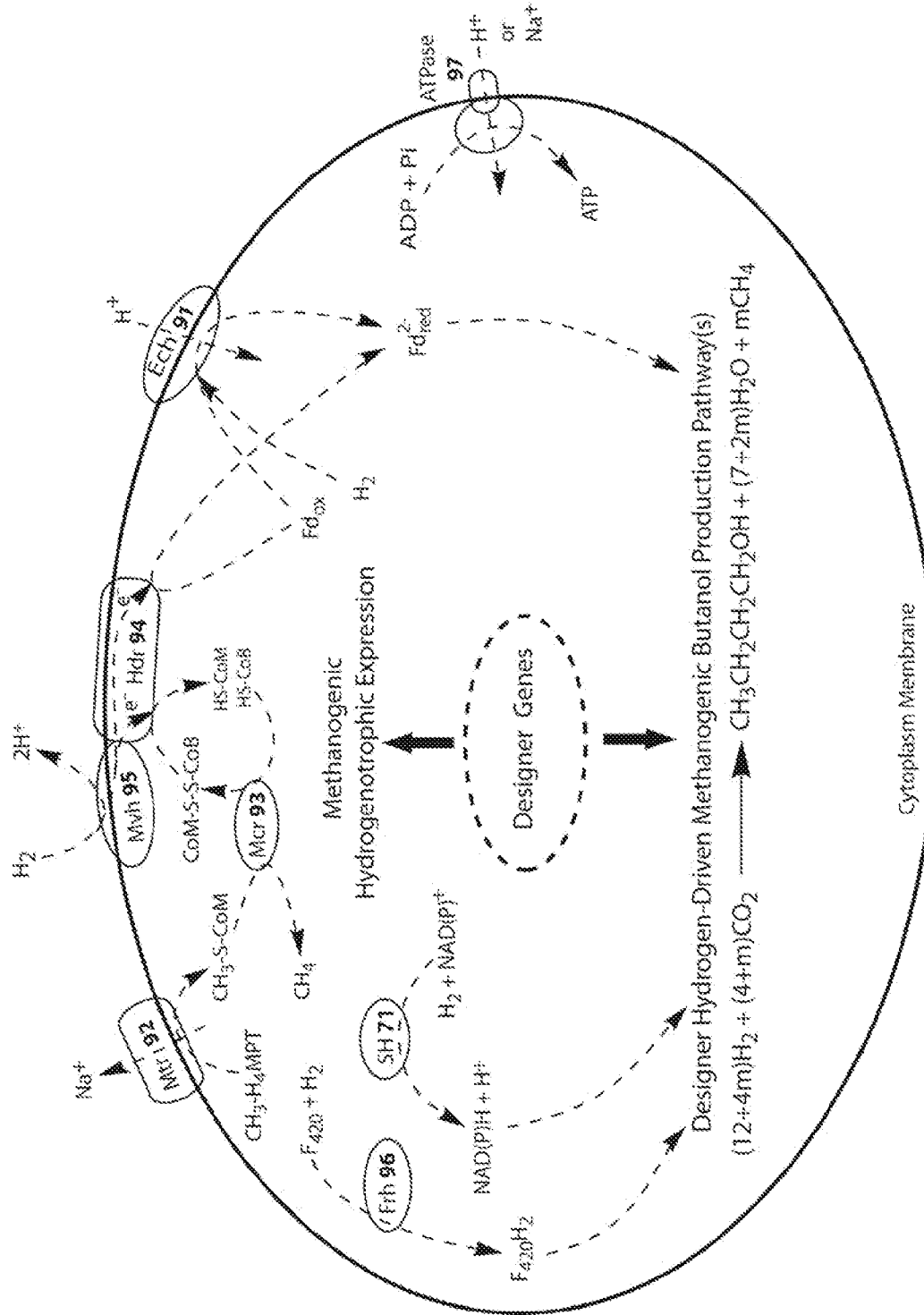


FIG. 15

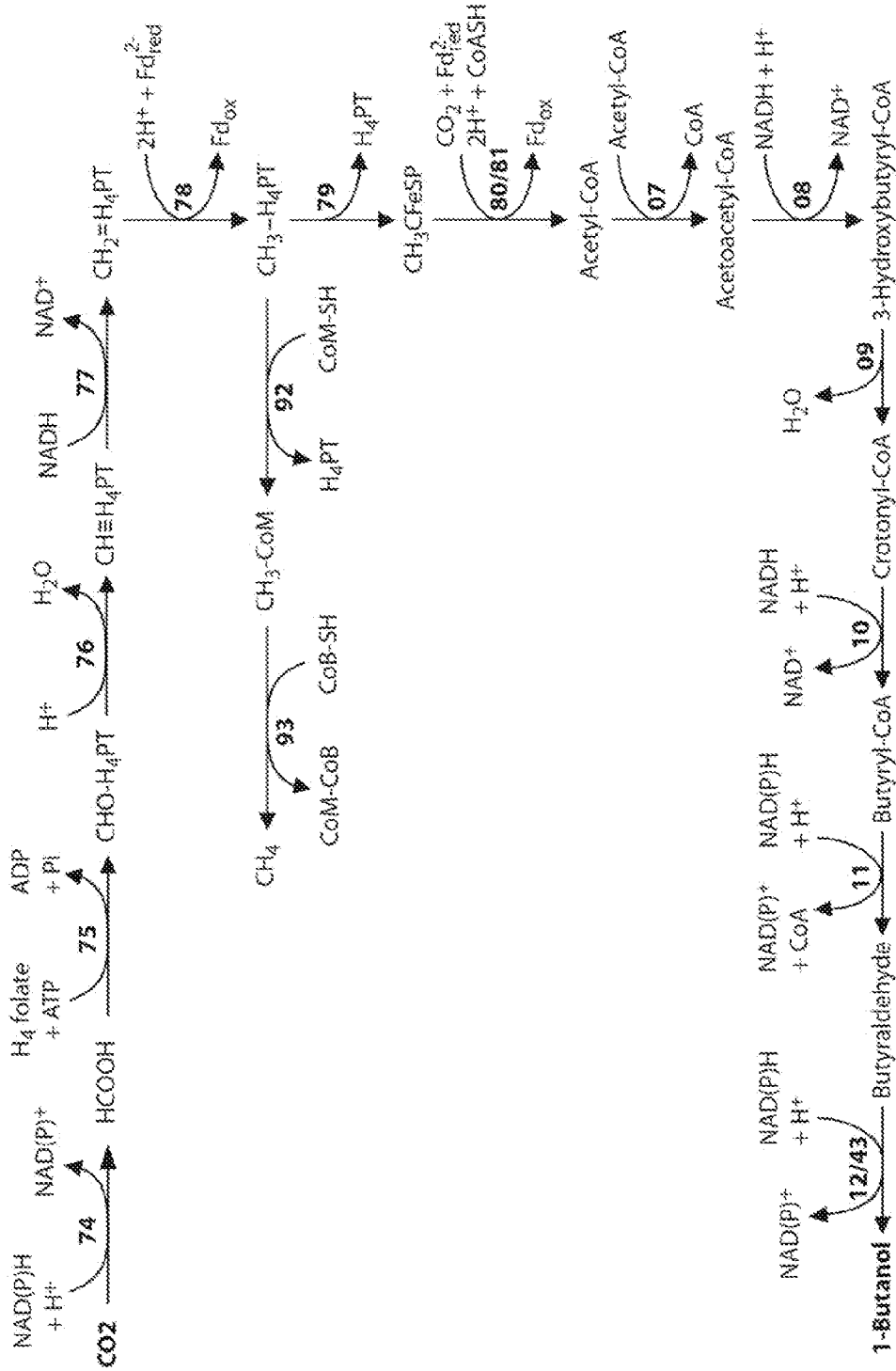


FIG. 16

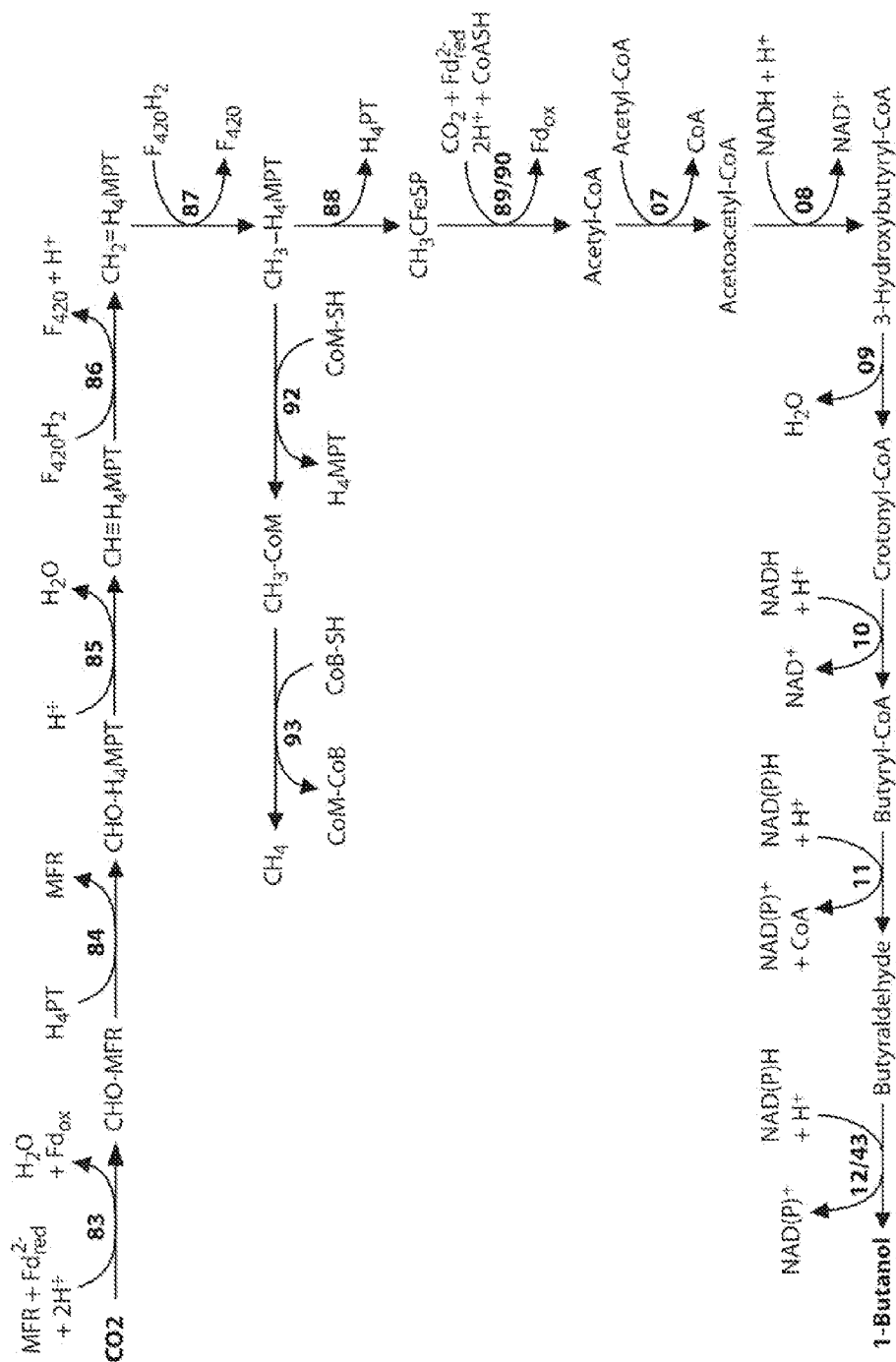


FIG. 17

**DESIGNER CALVIN-CYCLE-CHANNELED
AND HYDROGENOTROPHIC PRODUCTION
OF BUTANOL AND RELATED HIGHER
ALCOHOLS**

CROSS-REFERENCE TO RELATED
APPLICATIONS

[0001] This application claims the benefit of U.S. patent application Ser. No. 13/075,153 filed on Mar. 29, 2011, which is a continuation-in-part of co-pending U.S. patent application Ser. No. 12/918,784 filed on Aug. 20, 2010, which is the National Stage of International Application No. PCT/US2009/034801 filed on Feb. 21, 2009, which claims the benefit of U.S. Provisional Application No. 61/066,845 filed on Feb. 23, 2008, and U.S. Provisional Application No. 61/066,835 filed on Feb. 23, 2008. This application also claims the benefit of U.S. Provisional Application No. 61/426,147 filed on Dec. 22, 2010. The entire disclosures of all of these applications are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention generally relates to biosafety-guarded biofuel energy production technology. More specifically, the present invention provides an autotrophic advanced-biofuels production methodology based on designer transgenic plants, such as transgenic algae, blue-green algae (cyanobacteria and oxychlorobacteria), plant cells or bacterial cells that are created to use the reducing power (NADPH) or Hydrogen (H₂), and energy (ATP) acquired from the photosynthetic and/or hydrogenotrophic process for autotrophic synthesis of butanol and/or related higher alcohols from carbon dioxide (CO₂) and water (H₂O).

REFERENCE TO SEQUENCE LISTING

[0003] The present invention contains references to amino acid sequences and/or nucleic acid sequences which have been submitted concurrently herewith as the sequence listing text file "JWL_004_PCT_SeqListingFull_ST25.txt" updated on Dec. 18, 2011 from the efile of "JWL_004_US1_SeqListingFull_ST25.txt", file size 429 KB, created on Mar. 29, 2011, in electronic format using the Electronic Filing System of the U.S. Patent and Trademark Office. The aforementioned sequence listing was prepared with PatentIn 3.5, which complies with all format requirements specified in World Intellectual Property Organization Standard (WIPO) ST.25 and the related United States (US) final rule, and is incorporated herein by reference in its entirety including pursuant to 37 C.F.R. §1.52(e)(5) where applicable.

BACKGROUND OF THE INVENTION

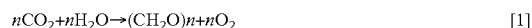
[0004] Butanol and/or related higher alcohols can be used as a liquid fuel to run engines such as cars. Butanol can replace gasoline and the energy contents of the two fuels are nearly the same (110,000 Btu per gallon for butanol; 115,000 Btu per gallon for gasoline). Butanol has many superior properties as an alternative fuel when compared to ethanol as well. These include: 1) Butanol has higher energy content (110,000 Btu per gallon butanol) than ethanol (84,000 Btu per gallon ethanol); 2) Butanol is six times less "evaporative" than ethanol and 13.5 times less evaporative than gasoline, making it safer to use as an oxygenate and thereby eliminating the need for very special blends during the summer and winter seasons; 3) Butanol can be transported through the existing fuel

infrastructure including the gasoline pipelines whereas ethanol must be shipped via rail, barge or truck; and 4) Butanol can be used as replacement for gasoline gallon for gallon e.g. 100% or any other percentage, whereas ethanol can only be used as an additive to gasoline up to about 85% (E-85) and then only after significant modification to the engine (while butanol can work as a 100% replacement fuel without having to modify the current car engine).

[0005] A significant potential market for butanol and/or related higher alcohols as a liquid fuel already exists in the current transportation and energy systems. Butanol is also used as an industrial solvent. In the United States, currently, butanol is manufactured primarily from petroleum. Historically (1900s-1950s), biobutanol was manufactured from corn and molasses in a fermentation process that also produced acetone and ethanol and was known as an ABE (acetone, butanol, ethanol) fermentation typically with certain butanol-producing bacteria such as *Clostridium acetobutylicum* and *Clostridium beijerinckii*. When the USA lost its low-cost sugar supply from Cuba around 1954, however, butanol production by fermentation declined mainly because the price of petroleum dropped below that of sugar. Recently, there is renewed R&D interest in producing butanol and/or ethanol from biomass such as corn starch using Clostridia- and/or yeast-fermentation process. However, similarly to the situation of "cornstarch ethanol production," the "cornstarch butanol production" process also requires a number of energy-consuming steps including agricultural corn-crop cultivation, corn-grain harvesting, corn-grain starch processing, and starch-to-sugar-to-butanol fermentation. The "cornstarch butanol production" process could also probably cost nearly as much energy as the energy value of its product butanol. This is not surprising, understandably because the cornstarch that the current technology can use represents only a small fraction of the corn crop biomass that includes the corn stalks, leaves and roots. The cornstovers are commonly discarded in the agricultural fields where they slowly decompose back to CO₂, because they represent largely lignocellulosic biomass materials that the current biorefinery industry cannot efficiently use for ethanol or butanol production. There are research efforts in trying to make ethanol or butanol from lignocellulosic plant biomass materials—a concept called "cellulosic ethanol" or "cellulosic butanol". However, plant biomass has evolved effective mechanisms for resisting assault on its cell-wall structural sugars from the microbial and animal kingdoms. This property underlies a natural recalcitrance, creating roadblocks to the cost-effective transformation of lignocellulosic biomass to fermentable sugars. Therefore, one of its problems known as the "lignocellulosic recalcitrance" represents a formidable technical barrier to the cost-effective conversion of plant biomass to fermentable sugars. That is, because of the recalcitrance problem, lignocellulosic biomasses (such as cornstover, switchgrass, and woody plant materials) could not be readily converted to fermentable sugars to make ethanol or butanol without certain pretreatment, which is often associated with high processing cost. Despite more than 50 years of R&D efforts in lignocellulosic biomass pretreatment and fermentative butanol-production processing, the problem of recalcitrant lignocellulosics still remains as a formidable technical barrier that has not yet been eliminated so far. Furthermore, the steps of lignocellulosic biomass cultivation, harvesting, pretreatment processing, and cellulose-to-sugar-to-butanol fermentation

all cost energy. Therefore, any new technology that could bypass these bottleneck problems of the biomass technology would be useful.

[0006] Oxyphotobacteria (also known as blue-green algae including cyanobacteria and oxychlorobacteria) and algae (such as *Chlamydomonas reinhardtii*, *Platymonas subcordiformis*, *Chlorella fusca*, *Dunaliella salina*, *Ankistrodesmus braunii*, and *Scenedesmus obliquus*), which can perform photosynthetic assimilation of CO₂ with O₂ evolution from water in a liquid culture medium with a maximal theoretical solar-to-biomass energy conversion of about 10%, have tremendous potential to be a clean and renewable energy resource. However, the wild-type oxygenic photosynthetic green plants, such as blue-green algae and eukaryotic algae, do not possess the ability to produce butanol directly from CO₂ and H₂O. The wild-type photosynthesis uses the reducing power (NADPH) and energy (ATP) from the photosynthetic water splitting and proton gradient-coupled electron transport process through the algal thylakoid membrane system to reduce CO₂ into carbohydrates (CH₂O)_n, such as starch with a series of enzymes collectively called the "Calvin cycle" at the stroma region in an algal or green-plant chloroplast. The net result of the wild-type photosynthetic process is the conversion of CO₂ and H₂O into carbohydrates (CH₂O)_n and O₂ using sunlight energy according to the following process reaction:



The carbohydrates (CH₂O)_n are then further converted to all kinds of complicated cellular (biomass) materials including proteins, lipids, and cellulose and other cell-wall materials during cell metabolism and growth.

[0007] In certain alga such as *Chlamydomonas reinhardtii*, some of the organic reserves such as starch could be slowly metabolized to ethanol (but not to butanol) through a secondary fermentative metabolic pathway. The algal fermentative metabolic pathway is similar to the yeast-fermentation process, by which starch is breakdown to smaller sugars such as glucose that is, in turn, transformed into pyruvate by a glycolysis process. Pyruvate may then be converted to formate, acetate, and ethanol by a number of additional metabolic steps (Gfeller and Gibbs (1984) "Fermentative metabolism of *Chlamydomonas reinhardtii*," *Plant Physiol.* 75:212-218). The efficiency of this secondary metabolic process is quite limited, probably because it could use only a small fraction of the limited organic reserve such as starch in an algal cell. Furthermore, the native algal secondary metabolic process could not produce any butanol. As mentioned above, butanol (and/or related higher alcohols) has many superior physical properties to serve as a replacement for gasoline as a fuel. Therefore, a new photobiological and/or hydrogenotrophic butanol (and/or related higher alcohols)-producing mechanism with a high energy conversion efficiency is needed.

[0008] International Application No. PCT/US2009/034801 discloses a set of methods on designer photosynthetic organisms (such as designer transgenic plant, plant cells, algae and oxyphotobacteria) for photobiological production of butanol from carbon dioxide (CO₂) and water (H₂O).

SUMMARY OF THE INVENTION

[0009] The present invention discloses designer Calvin-cycle-channeled and/or hydrogenotrophic pathways, the associated designer genes and designer transgenic photosynthetic organisms for autotrophic production of butanol and/or

related higher alcohols that are selected from the group that consists of: 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, 6-methyl-1-heptanol, and combinations thereof.

[0010] The designer autotrophic organisms such as designer transgenic oxyphotobacteria and algae comprise designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathway gene(s) and biosafety-guarding technology for enhanced photobiological production of butanol and related higher alcohols from carbon dioxide and water.

[0011] According to another embodiment, the transgenic autotrophic organism comprises a transgenic designer plant or plant cells selected from the group consisting of aquatic plants, plant cells, green algae, red algae, brown algae, blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria), diatoms, marine algae, freshwater algae, salt-tolerant algal strains, cold-tolerant algal strains, heat-tolerant algal strains, antenna-pigment-deficient mutants, butanol-tolerant algal strains, higher-alcohols-tolerant algal strains, butanol-tolerant oxyphotobacteria, higher-alcohols-tolerant oxyphotobacteria, and combinations thereof.

[0012] According to one of the various embodiments, a designer Calvin-cycle-channeled photosynthetic NADPH-enhanced pathway that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 1-butanol comprises a set of enzymes selected from the group consisting of: NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, 2-isopropylmalate synthase, isopropylmalate isomerase, 2-keto acid decarboxylase, alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and butanol dehydrogenase.

[0013] According to one of the various embodiments, another designer Calvin-cycle-channeled photosynthetic NADPH-enhanced 1-butanol-production pathway comprises a set of enzymes selected from the group consisting of: NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, 2-isopropylmalate synthase, isopropylmalate isomerase, 3-isopropylmalate dehydrogenase, 2-keto acid decarboxylase, and NAD-dependent alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and butanol dehydrogenase.

[0014] According to another embodiment, a designer Calvin-cycle-channeled photosynthetic NADPH-enhanced pathway that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 2-methyl-1-butanol, comprises a set of enzymes selected from the group consisting of: NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, acetolactate synthase, ketol-acid reductoi-

somerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, NAD-dependent alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and 2-methylbutyraldehyde reductase.

[0015] According to another embodiment, a designer Calvin-cycle-channeled photosynthetic NADPH-enhanced pathway for photobiological production of 2-methyl-1-butanol production comprises a set of enzymes selected from the group consisting of: NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, and NAD dependent alcohol dehydrogenase, NADPH dependent alcohol dehydrogenase, and 2-methylbutyraldehyde reductase.

[0016] According to another embodiment, a designer Calvin-cycle-channeled photosynthetic NADPH-enhanced pathway for photobiological production of isobutanol comprises a set of enzymes selected from the group consisting of: NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, and NAD-dependent alcohol dehydrogenase, and NADPH-dependent alcohol dehydrogenase.

[0017] Likewise, a number of other designer Calvin-cycle-channeled photosynthetic NADPH-enhanced pathways are also disclosed according to one of the various embodiments for photobiological production of butanol and/or related higher alcohols such as 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, and/or 6-methyl-1-heptanol.

[0018] According to one of various embodiments, a method for photobiological production and harvesting of butanol and related higher alcohols comprises: a) introducing a transgenic photosynthetic organism into a photobiological reactor system, the transgenic photosynthetic organism comprising transgenes coding for a set of enzymes configured to act on an intermediate product of a Calvin cycle and to convert the intermediate product into butanol and/or related higher alcohols; b) using reducing power NADPH and energy ATP associated with the transgenic photosynthetic organism acquired from photosynthetic water splitting and proton gradient coupled electron transport process in the photobioreactor to synthesize butanol and/or related higher alcohols from carbon dioxide and water; and c) using a product separation process to harvest the synthesized butanol and/or related higher alcohols from the photobioreactor.

[0019] According to another embodiment, designer hydrogen-driven Calvin-cycle-channeled biofuel-production organisms for chemolithoautotrophic production of butanol and related higher alcohols comprises a set of oxygen-tolerant soluble hydrogenase and membrane-bound hydrogenases in combination with the designer Calvin-cycle-channeled biofuel-production pathways.

[0020] According to another embodiment, a designer organism comprises a designer anaerobic hydrogenotrophic system and a reductive-acetyl-CoA biofuel-production path-

way(s) for hydrogen-driven chemolithoautotrophic production of 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from hydrogen (H_2) and carbon dioxide (CO_2) with its maximal H_2 -to-butanol energy conversion efficiency as high as 91%. This designer autotrophic organism comprises a set of designer genes (e.g., designer DNA constructs) that express the designer anaerobic hydrogenotrophic butanol-production-pathway system comprising: energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F_{420} -reducing hydrogenase (Frh), native (or heterologous) soluble hydrogenase (SH), heterodisulfide reductase (Hdr), formylmethanofuran dehydrogenase, formyl transferase, 10-methenyl-tetrahydromethanopterin cyclohydrolase, 10-methylene- H_4 methanopterin dehydrogenase, 10-methylene- H_4 -methanopterin reductase, methyl- H_4 -methanopterin: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, butanol dehydrogenase and/or alcohol dehydrogenase.

[0021] According to one of the various embodiments, a designer autotrophic organism comprises a designer methanogenic hydrogenotrophic system and a reductive-acetyl-CoA biofuel-production pathway(s) for anaerobic chemolithoautotrophic production of both 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and methane (CH_4) from hydrogen (H_2) and carbon dioxide (CO_2). This designer autotrophic organism comprises a set of designer genes that express a designer methanogenic hydrogenotrophic butanol-production-pathway system comprising: methyl-H4MPT: coenzyme-M methyltransferase Mtr, native (or heterologous) A_1A_2 -ATP synthase, methyl-coenzyme M reductase Mcr, energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F_{420} -reducing hydrogenase (Frh), soluble hydrogenase (SH), heterodisulfide reductase (Hdr), formate dehydrogenase, 10-formyl- H_4 folate synthetase, methenyltetrahydrofolate cyclohydrolase, 10-methylene- H_4 folate dehydrogenase, 10-methylene- H_4 folate reductase, methyl- H_4 folate: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase and/or alcohol dehydrogenase.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 presents designer butanol-production pathways branched from the Calvin cycle using the reducing power (NADPH) and energy (ATP) from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into butanol $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ with a series of enzymatic reactions.

[0023] FIG. 2A presents a DNA construct for designer butanol-production-pathway gene(s).

[0024] FIG. 2B presents a DNA construct for NADPH/NADH-conversion designer gene for NADPH/NADH interconversion.

[0025] FIG. 2C presents a DNA construct for a designer iRNA starch/glycogen-synthesis inhibitor(s) gene.

[0026] FIG. 2D presents a DNA construct for a designer starch-degradation-glycolysis gene(s).

[0027] FIG. 2E presents a DNA construct of a designer butanol-production-pathway gene(s) for cytosolic expression.

[0028] FIG. 2F presents a DNA construct of a designer butanol-production-pathway gene(s) with two recombination sites for integrative genetic transformation in oxyphotobacteria.

[0029] FIG. 2G presents a DNA construct of a designer biosafety-control gene(s).

[0030] FIG. 2H presents a DNA construct of a designer proton-channel gene(s).

[0031] FIG. 3A illustrates a cell-division-controllable designer organism that contains two key functions: designer biosafety mechanism(s) and designer biofuel-production pathway(s).

[0032] FIG. 3B illustrates a cell-division-controllable designer organism for photobiological production of butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) with designer biosafety mechanism(s).

[0033] FIG. 3C illustrates a cell-division-controllable designer organism for biosafety-guarded photobiological production of other biofuels such as ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O).

[0034] FIG. 4 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using the reducing power (NADPH) and energy (ATP) from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0035] FIG. 5 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 2-methyl-1-butanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0036] FIG. 6 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into isobutanol ($(\text{CH}_3)_2\text{CHCH}_2\text{OH}$) and 3-methyl-1-butanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0037] FIG. 7 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 1-hexanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and 1-octanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0038] FIG. 8 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 1-pentanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 1-hexanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), and 1-heptanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0039] FIG. 9 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 3-methyl-1-pentanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$), 4-methyl-1-hexanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)$

$\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), and 5-methyl-1-heptanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0040] FIG. 10 presents designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways using NADPH and ATP from the photosynthetic water splitting and proton gradient-coupled electron transport process to reduce carbon dioxide (CO_2) into 4-methyl-1-pentanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 5-methyl-1-hexanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), and 6-methyl-1-heptanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) with a series of enzymatic reactions.

[0041] FIG. 11 illustrates a designer organism with designer oxygen-tolerant hydrogenases and Calvin-cycle-channeled biofuel-production pathway(s) for aerobic chemolithoautotrophic production of biofuels such as butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from hydrogen (H_2), carbon dioxide (CO_2), and oxygen (O_2).

[0042] FIG. 12 illustrates a designer organism that comprises a designer anaerobic hydrogenotrophic system with reductive-acetyl-CoA biofuel-production pathway(s) for anaerobic chemolithotrophic production of 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from hydrogen (H_2) and carbon dioxide (CO_2).

[0043] FIG. 13 presents a designer reductive-acetyl-CoA biofuel-production pathway for anaerobic hydrogenotrophic production of 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) with a series of enzymatic reactions.

[0044] FIG. 14 presents a designer ATP-required reductive-acetyl-CoA biofuel-production pathway for anaerobic hydrogenotrophic production of 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) with a series of enzymatic reactions.

[0045] FIG. 15 illustrates a designer organism that comprises a designer methanogenic hydrogenotrophic system with reductive-acetyl-CoA biofuel-production pathway(s) for anaerobic chemolithotrophic production of both 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and methane (CH_4) from hydrogen (H_2) and carbon dioxide (CO_2).

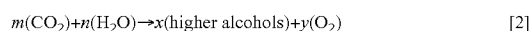
[0046] FIG. 16 presents designer reductive-acetyl-CoA biofuel-production pathways for anaerobic hydrogenotrophic production of both 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and methane (CH_4) from carbon dioxide (CO_2) with a series of enzymatic reactions.

[0047] FIG. 17 presents designer ATP-required reductive-acetyl-CoA biofuel-production pathways for anaerobic hydrogenotrophic production of both 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and methane (CH_4) from carbon dioxide (CO_2) and with a series of enzymatic reactions.

DETAILED DESCRIPTION OF THE INVENTION

[0048] The present invention is directed to an autotrophic butanol and related high alcohols production technology based on designer autotrophic organisms such as designer transgenic plants (e.g., algae and oxyphotobacteria), plant cells, or bacteria. In this context throughout this specification, a "higher alcohol" or "related higher alcohol" refers to an alcohol that comprises at least four carbon atoms, which includes both straight and branched alcohols such as 1-butanol and 2-methyl-1-butanol. The Calvin-cycle-channeled and photosynthetic-NADPH-enhanced pathways are constructed with designer enzymes expressed through use of designer genes in host photosynthetic organisms such as algae and oxyphotobacteria (including cyanobacteria and

oxychlorobacteria) organisms for photobiological production of butanol and related higher alcohols. The said butanol and related higher alcohols are selected from the group consisting of: 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, and 6-methyl-1-heptanol. The designer plants and plant cells are created using genetic engineering techniques such that the endogenous photosynthesis regulation mechanism is tamed, and the reducing power (NADPH) and energy (ATP) acquired from the photosynthetic water splitting and proton gradient-coupled electron transport process can be used for immediate synthesis of higher alcohols, such as 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) and 2-methyl-1-butanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{OH}$), from carbon dioxide (CO_2) and water (H_2O) according to the following generalized process reaction (where m, n, x and y are its molar coefficients) in accordance of the present invention:



The photobiological higher-alcohols-production methods of the present invention completely eliminate the problem of recalcitrant lignocellulosics by bypassing the bottleneck problem of the biomass technology. As shown in FIG. 1, for example, the photosynthetic process in a designer organism effectively uses the reducing power (NADPH) and energy (ATP) from the photosynthetic water splitting and proton gradient-coupled electron transport process for immediate synthesis of butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) directly from carbon dioxide (CO_2) and water (H_2O) without being drained into the other pathway for synthesis of the undesirable lignocellulosic materials that are very hard and often inefficient for the biorefinery industry to use. This approach is also different from the existing "cornstarch butanol production" process. In accordance with this invention, butanol can be produced directly from carbon dioxide (CO_2) and water (H_2O) without having to go through many of the energy consuming steps that the cornstarch butanol-production process has to go through, including corn crop cultivation, corn-grain harvesting, corn-grain cornstarch processing, and starch-to-sugar-to-butanol fermentation. As a result, the photosynthetic butanol-production technology of the present invention is expected to have a much (more than 10-times) higher solar-to-butanol energy-conversion efficiency than the current technology. Assuming a 10% solar energy conversion efficiency for the proposed photosynthetic butanol production process, the maximal theoretical productivity (yield) could be about 72,700 kg of butanol per acre per year, which could support about 70 cars (per year per acre). Therefore, this invention could bring a significant capability to the society in helping to ensure energy security. The present invention could also help protect the Earth's environment from the dangerous accumulation of CO_2 in the atmosphere, because the present methods convert CO_2 directly into clean butanol energy.

[0049] A fundamental feature of the present methodology is utilizing a plant (e.g., an alga or oxyphotobacterium) or plant cells, introducing into the plant or plant cells nucleic acid molecules encoding for a set of enzymes that can act on an intermediate product of the Calvin cycle and convert the intermediate product into butanol as illustrated in FIG. 1, instead of making starch and other complicated cellular (biomass) materials as the end products by the wild-type photosynthetic pathway. Accordingly, the present invention pro-

vides, inter alia, methods for producing butanol and/or related higher alcohols based on a designer plant (such as a designer alga and a designer oxyphotobacterium), designer plant tissue, or designer plant cells, DNA constructs encoding genes of a designer butanol- and/or related higher alcohols-production pathway(s), as well as the designer algae, designer oxyphotobacteria (including designer cyanobacteria), designer plants, designer plant tissues, and designer plant cells created. The various aspects of the present invention are described in further detail hereinbelow.

Host Photosynthetic Organisms

[0050] According to the present invention, a designer organism or cell for the photosynthetic butanol and/or related higher alcohols production of the invention can be created utilizing as host, any plant (including alga and oxyphotobacterium), plant tissue, or plant cells that have a photosynthetic capability, i.e., an active photosynthetic apparatus and enzymatic pathway that captures light energy through photosynthesis, using this energy to convert inorganic substances into organic matter. Preferably, the host organism should have an adequate photosynthetic CO_2 fixation rate, for example, to support photosynthetic butanol (and/or related higher alcohols) production from CO_2 and H_2O at least about 1,450 kg butanol per acre per year, more preferably, 7,270 kg butanol per acre per year, or even more preferably, 72,700 kg butanol per acre per year.

[0051] In a preferred embodiment, an aquatic plant is utilized to create a designer plant. Aquatic plants, also called hydrophytic plants, are plants that live in or on aquatic environments, such as in water (including on or under the water surface) or permanently saturated soil. As used herein, aquatic plants include, for example, algae, blue-green algae (cyanobacteria and oxychlorobacteria), submersed aquatic herbs (*Hydrilla verticillata*, *Elodea densa*, *Hippuris vulgaris*, *Aponogeton Boivinianus*, *Aponogeton Rigidifolius*, *Aponogeton Longiplumulosus*, *Didiplis Diandra*, *Vesicularia Dubyana*, *Hygrophila Augustifolia*, *Micranthemum Umbrosum*, *Eichhornia Azurea*, *Saururus Cernuus*, *Cryptocoryne Lingua*, *Hydrotriche Hottoniiflora Eustralis Stellata*, *Vallisneria Rubra*, *Hygrophila Salicifolia*, *Cyperus Helferi*, *Cryptocoryne Petchii*, *Vallisneria americana*, *Vallisneria Torta*, *Hydrotriche Hottoniiflora*, *Crassula Helmsii*, *Limnophila Sessiliflora*, *Potamogeton Perfoliatus*, *Rotala Wallichii*, *Cryptocoryne Becketii*, *Blyxa Aubertii*, *Hygrophila Difformis*), duckweeds (*Spirodela polyrrhiza*, *Wolffia globosa*, *Lemna trisulca*, *Lemna gibba*, *Lemna minor*, *Landoltia punctata*), water cabbage (*Pistia stratiotes*), buttercups (*Ranunculus*), water caltrop (*Trapa natans* and *Trapa bicornis*), water lily (*Nymphaea lotus*, *Nymphaeaceae* and *Nelumbonaceae*), water hyacinth (*Eichhornia crassipes*), *Bolbitis heudelotii*, *Cabomba* sp., seagrasses (*Heteranthera Zosterifolia*, *Posidoniaceae*, *Zosteraceae*, *Hydrocharitaceae*, and *Cymodoceaceae*). Butanol (and/or related higher alcohols) produced from an aquatic plant can diffuse into water, permitting normal growth of the plants and more robust production of butanol from the plants. Liquid cultures of aquatic plant tissues (including, but not limited to, multicellular algae) or cells (including, but not limited to, unicellular algae) are also highly preferred for use, since the butanol (and/or related higher alcohols) molecules produced from a designer butanol (and/or related higher alcohols) production pathway(s) can readily diffuse out of the cells or tissues into the liquid water medium, which can serve as a large pool to store the product

butanol (and/or related higher alcohols) that can be subsequently harvested by filtration and/or distillation/evaporation techniques.

[0052] Although aquatic plants or cells are preferred host organisms for use in the methods of the present invention, tissue and cells of non-aquatic plants, which are photosynthetic and can be cultured in a liquid culture medium, can also be used to create designer tissue or cells for photosynthetic butanol (and/or related higher alcohols) production. For example, the following tissue or cells of non-aquatic plants can also be selected for use as a host organism in this invention: the photoautotrophic shoot tissue culture of wood apple tree *Feronia limonia*, the chlorophyllous callus-cultures of corn plant *Zea mays*, the green root cultures of Asteraceae and Solanaceae species, the tissue culture of sugarcane stalk parenchyma, the tissue culture of bryophyte *Physcomitrella patens*, the photosynthetic cell suspension cultures of soybean plant (*Glycine max*), the photoautotrophic and photomixotrophic culture of green Tobacco (*Nicotiana tabacum* L.) cells, the cell suspension culture of *Gisekia pharmaceoides* (a C₄ plant), the photosynthetic suspension cultured lines of *Amaranthus powellii* Wats., *Datura innoxia* Mill., *Gossypium hirsutum* L., and *Nicotiana tabacum* × *Nicotiana glutinosa* L. fusion hybrid.

[0053] By “liquid medium” is meant liquid water plus relatively small amounts of inorganic nutrients (e.g., N, P, K etc, commonly in their salt forms) for photoautotrophic cultures; and sometimes also including certain organic substrates (e.g., sucrose, glucose, or acetate) for photomixotrophic and/or photoheterotrophic cultures.

[0054] In an especially preferred embodiment, the plant utilized in the butanol (and/or related higher alcohols) production method of the present invention is an alga or a blue-green alga. The use of algae and/or blue-green algae has several advantages. They can be grown in an open pond at large amounts and low costs. Harvest and purification of butanol (and/or related higher alcohols) from the water phase is also easily accomplished by distillation/evaporation or membrane separation.

[0055] Algae suitable for use in the present invention include both unicellular algae and multi-unicellular algae. Multicellular algae that can be selected for use in this invention include, but are not limited to, seaweeds such as *Ulva latissima* (sea lettuce), *Ascophyllum nodosum*, *Codium fragile*, *Fucus vesiculosus*, *Eucheuma denticulatum*, *Gracilaria gracilis*, *Hydrodictyon reticulatum*, *Laminaria japonica*, *Undaria pinnatifida*, *Saccharina japonica*, *Porphyra yezoensis*, and *Porphyra tenera*. Suitable algae can also be chosen from the following divisions of algae: green algae (Chlorophyta), red algae (Rhodophyta), brown algae (Phaeophyta), diatoms (Bacillariophyta), and blue-green algae (Oxyphotobacteria including Cyanophyta and Prochlorophytes). Suitable orders of green algae include Ulvales, Ulotrichales, Volvocales, Chlorellales, Schizogoniales, Oedogoniales, Zygnematales, Cladophorales, Siphonales, and Dasycladales. Suitable genera of Rhodophyta are *Porphyra*, *Chondrus*, *Cyanidioschyzon*, *Porphyridium*, *Gracilaria*, *Kappaphycus*, *Gelidium* and *Agardhiella*. Suitable genera of Phaeophyta are *Laminaria*, *Undaria*, *Macrocystis*, *Sargassum* and *Dictyosiphon*. Suitable genera of Cyanophyta (also known as Cyanobacteria) include (but not limited to) *Phoridium*, *Synechocystis*, *Synechococcus*, *Oscillatoria*, and *Anabaena*. Suitable genera of Prochlorophytes (also known as oxychlorobacteria) include (but not limited to) *Prochloron*, *Prochlorothrix*, and

Prochlorococcus. Suitable genera of Bacillariophyta are *Cyclotella*, *Cylindrotheca*, *Navicula*, *Thalassiosira*, and *Phaeodactylum*. Preferred species of algae for use in the present invention include *Chlamydomonas reinhardtii*, *Platymonas subcordiformis*, *Chlorella fusca*, *Chlorella sorokiniana*, *Chlorella vulgaris*, ‘*Chlorella*’ *ellipsoidea*, *Chlorella* spp., *Dunaliella salina*, *Dunaliella viridis*, *Dunaliella bardowil*, *Haematococcus pluvialis*; *Parachlorella kessleri*, *Betaphycus gelatinum*, *Chondrus crispus*, *Cyanidioschyzon merolae*, *Cyanidium caldarium*, *Galdieria sulphuraria*, *Gelidiella acerosa*, *Gracilaria changii*, *Kappaphycus alvarezii*, *Porphyra miniata*, *Ostreococcus tauri*, *Porphyra yezoensis*, *Porphyridium* sp., *Palmaria palmata*, *Gracilaria* spp., *Isochrysis galbana*, *Kappaphycus* spp., *Laminaria japonica*, *Laminaria* spp., *Monostroma* spp., *Nannochloropsis oculata*, *Porphyra* spp., *Porphyridium* spp., *Undaria pinnatifida*, *Ulva lactuca*, *Ulva* spp., *Undaria* spp., *Phaeodactylum Tricornutum*, *Navicula saprophila*, *Cryptocodinium cohnii*, *Cylindrotheca fusiformis*, *Cyclotella cryptica*, *Euglena gracilis*, *Amphidinium* sp., *Symbiodinium microadriaticum*, *Macrocystis pyrifera*, *Ankistrodesmus braunii*, and *Scenedesmus obliquus*.

[0056] Preferred species of blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria) for use in the present invention include *Thermosynechococcus elongatus* BP-1, *Nostoc* sp. PCC 7120, *Synechococcus elongatus* PCC 6301, *Synechococcus* sp. strain PCC 7942, *Synechococcus* sp. strain PCC 7002, *Synechocystis* sp. strain PCC 6803, *Prochlorococcus marinus* MED4, *Prochlorococcus marinus* MIT 9313, *Prochlorococcus marinus* NATL1A, *Prochlorococcus* SS120, *Spirulina platensis* (*Arthrospira platensis*), *Spirulina pacifica*, *Lyngbya majuscula*, *Anabaena* sp., *Synechocystis* sp., *Synechococcus elongates*, *Synechococcus* (MC-A), *Trichodesmium* sp., *Richelia intracellularis*, *Synechococcus* WH7803, *Synechococcus* WH8102, *Nostoc punctiforme*, *Synechococcus* sp. strain PCC 7943, *Synechocystis* PCC 6714 phycocyanin-deficient mutant PD-1, *Cyanothece* strain 51142, *Cyanothece* sp. CCY0110, *Oscillatoria limosa*, *Lyngbya majuscula*, *Symploca muscorum*, *Gloeobacter violaceus*, *Prochloron didemni*, *Prochlorothrix hollandica*, *Synechococcus* (MC-A), *Trichodesmium* sp., *Richelia intracellularis*, *Prochlorococcus marinus*, *Prochlorococcus* SS120, *Synechococcus* WH8102, *Lyngbya majuscula*, *Symploca muscorum*, *Synechococcus bigranulatus*, cryophilic *Oscillatoria* sp., *Phormidium* sp., *Nostoc* sp.-1, *Calothrix parietina*, thermophilic *Synechococcus bigranulatus*, *Synechococcus lividus*, thermophilic *Mastigocladus laminosus*, *Chlorogloeopsis fritschii* PCC 6912, *Synechococcus vulcanus*, *Synechococcus* sp. strain MA4, *Synechococcus* sp. strain MA19, and *Thermosynechococcus elongatus*.

[0057] Proper selection of host photosynthetic organisms for their genetic backgrounds and certain special features is also beneficial. For example, a photosynthetic-butanol-producing designer alga created from cryophilic algae (psychrophiles) that can grow in snow and ice, and/or from cold-tolerant host strains such as *Chlamydomonas* cold strain CCMG1619, which has been characterized as capable of performing photosynthetic water splitting as cold as 4° C. (Lee, Blankinship and Greenbaum (1995), “Temperature effect on production of hydrogen and oxygen by *Chlamydomonas* cold strain CCMP1619 and wild type 137c,” *Applied Biochemistry and Biotechnology* 51/52:379-386), permits photobiological butanol production even in cold seasons or regions such as Canada. Meanwhile, a designer alga

created from a thermophilic/thermotolerant photosynthetic organism such as thermophilic algae *Cyanidium caldarium* and *Galdieria sulphuraria* and/or thermophilic cyanobacteria (blue-green algae) such as *Thermosynechococcus elongatus* BP-1 and *Synechococcus bigranulatus* may permit the practice of this invention to be well extended into the hot seasons or areas such as Mexico and the Southwestern region of the United States including Nevada, California, Arizona, New Mexico and Texas, where the weather can often be hot. Furthermore, a photosynthetic-butanol-producing designer alga created from a marine alga, such as *Platymonas subcordiformis*, permits the practice of this invention using seawater, while the designer alga created from a freshwater alga such as *Chlamydomonas reinhardtii* can use freshwater. Additional optional features of a photosynthetic butanol (and/or related higher alcohols) producing designer alga include the benefits of reduced chlorophyll-antenna size, which has been demonstrated to provide higher photosynthetic productivity (Lee, Mets, and Greenbaum (2002). "Improvement of photosynthetic efficiency at high light intensity through reduction of chlorophyll antenna size," *Applied Biochemistry and Biotechnology*, 98-100: 37-48) and butanol-tolerance (and/or related higher alcohols-tolerance) that allows for more robust and efficient photosynthetic production of butanol (and/or related higher alcohols) from CO₂ and H₂O. By use of a phycoerythrin-deficient mutant of *Synechocystis* PCC 6714, it has been experimentally demonstrated that photoinhibition can be reduced also by reducing the content of light-harvesting pigments (Nakajima, Tsuzuki, and Ueda (1999) "Reduced photoinhibition of a phycoerythrin-deficient mutant of *Synechocystis* PCC 6714", *Journal of Applied Phycology* 10: 447-452). These optional features can be incorporated into a designer alga, for example, by use of a butanol-tolerant and/or chlorophyll antenna-deficient mutant (e.g., *Chlamydomonas reinhardtii* strain DS521) as a host organism, for gene transformation with the designer butanol-production-pathway genes. Therefore, in one of the various embodiments, a host alga is selected from the group consisting of green algae, red algae, brown algae, blue-green algae (oxyphotobacteria including cyanobacteria and prochlorophytes), diatoms, marine algae, freshwater algae, unicellular algae, multicellular algae, seaweeds, cold-tolerant algal strains, heat-tolerant algal strains, light-harvesting-antenna-pigment-deficient mutants, butanol-tolerant algal strains, higher alcohols-tolerant algal strains, and combinations thereof.

Creating a Designer Butanol-Production Pathway in a Host

Selecting Appropriate Designer Enzymes

[0058] One of the key features in the present invention is the creation of a designer butanol-production pathway to tame and work with the natural photosynthetic mechanisms to achieve the desirable synthesis of butanol directly from CO₂ and H₂O. The natural photosynthetic mechanisms include (1) the process of photosynthetic water splitting and proton gradient-coupled electron transport through the thylakoid membrane, which produces the reducing power (NADPH) and energy (ATP), and (2) the Calvin cycle, which reduces CO₂ by consumption of the reducing power (NADPH) and energy (ATP).

[0059] In accordance with the present invention, a series of enzymes are used to create a designer butanol-production pathway that takes an intermediate product of the Calvin cycle and converts the intermediate product into butanol as

illustrated in FIG. 1. A "designer butanol-production-pathway enzyme" is hereby defined as an enzyme that serves as a catalyst for at least one of the steps in a designer butanol-production pathway. According to the present invention, a number of intermediate products of the Calvin cycle can be utilized to create designer butanol-production pathway(s); and the enzymes required for a designer butanol-production pathway are selected depending upon from which intermediate product of the Calvin cycle the designer butanol-production pathway branches off from the Calvin cycle.

[0060] In one example, a designer pathway is created that takes glyceraldehydes-3-phosphate and converts it into butanol by using, for example, a set of enzymes consisting of, as shown with the numerical labels 01-12 in FIG. 1, glyceraldehyde-3-phosphate dehydrogenase 01, phosphoglycerate kinase 02, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, pyruvate-ferredoxin oxidoreductase 06, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, and butanol dehydrogenase 12. In this glyceraldehydes-3-phosphate-branched designer pathway, for conversion of two molecules of glyceraldehyde-3-phosphate to butanol, two NADH molecules are generated from NAD⁺ at the step from glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate catalyzed by glyceraldehyde-3-phosphate dehydrogenase 01; meanwhile two molecules of NADH are converted to NAD⁺: one at the step catalyzed by 3-hydroxybutyryl-CoA dehydrogenase 08 in reducing acetoacetyl-CoA to 3-hydroxybutyryl-CoA and another at the step catalyzed by butyryl-CoA dehydrogenase 10 in reducing crotonyl-CoA to butyryl-CoA. Consequently, in this glyceraldehydes-3-phosphate-branched designer pathway (01-12), the number of NADH molecules consumed is balanced with the number of NADH molecules generated. Furthermore, both the pathway step catalyzed by butyraldehyde dehydrogenase 11 (in reducing butyryl-CoA to butyraldehyde) and the terminal step catalyzed by butanol dehydrogenase 12 (in reducing butyraldehyde to butanol) can use NADPH, which can be regenerated by the photosynthetic water splitting and proton gradient-coupled electron transport process. Therefore, this glyceraldehydes-3-phosphate-branched designer butanol-production pathway can operate continuously.

[0061] In another example, a designer pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 03-12 in FIG. 1) phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, pyruvate-ferredoxin oxidoreductase 06, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, and butanol dehydrogenase 12. It is worthwhile to note that the last ten enzymes (03-12) of the glyceraldehydes-3-phosphate-branched designer butanol-producing pathway (01-12) are identical with those utilized in the 3-phosphoglycerate-branched designer pathway (03-12). In other words, the designer enzymes (01-12) of the glyceraldehydes-3-phosphate-branched pathway permit butanol production from both the point of 3-phosphoglycerate and the point glyceraldehydes 3-phosphate in the Calvin cycle. These two pathways, however, have different characteristics. Unlike the glyceraldehyde-3-phosphate-branched butanol-production pathway, the 3-phosphoglycerate-branched pathway which consists of the activities of only ten enzymes (03-12) could not itself generate any NADH that is required for use at

two places: one at the step catalyzed by 3-hydroxybutyryl-CoA dehydrogenase 08 in reducing acetoacetyl-CoA to 3-hydroxybutyryl-CoA, and another at the step catalyzed by butyryl-CoA dehydrogenase 10 in reducing crotonyl-CoA to butyryl-CoA. That is, if (or when) a 3-hydroxybutyryl-CoA dehydrogenase and/or a butyryl-CoA dehydrogenase that can use strictly only NADH but not NADPH is employed, it would require a supply of NADH for the 3-phosphoglycerate-branched pathway (03-12) to operate. Consequently, in order for the 3-phosphoglycerate-branched butanol-production pathway to operate, it is important to use a 3-hydroxybutyryl-CoA dehydrogenase 08 and a butyryl-CoA dehydrogenase 10 that can use NADPH which can be supplied by the photo-driven electron transport process. Therefore, it is a preferred practice to use a 3-hydroxybutyryl-CoA dehydrogenase and a butyryl-CoA dehydrogenase that can use NADPH or both NADPH and NADH (i.e., NAD(P)H) for this 3-phosphoglycerate-branched designer butanol-production pathway (03-12 in FIG. 1). Alternatively, when a 3-hydroxybutyryl-CoA dehydrogenase and a butyryl-CoA dehydrogenase that can use only NADH are employed, it is preferably here to use an additional embodiment that can confer an NADPH/NADH conversion mechanism (to supply NADH by converting NADPH to NADH, see more detail later in the text) in the designer organism to facilitate photosynthetic production of butanol through the 3-phosphoglycerate-branched designer pathway.

[0062] In still another example, a designer pathway is created that takes fructose-1,6-diphosphate and converts it into butanol by using, as shown with the numerical labels 20-33 in FIG. 1, a set of enzymes consisting of aldolase 20, triose phosphate isomerase 21, glyceraldehyde-3-phosphate dehydrogenase 22, phosphoglycerate kinase 23, phosphoglycerate mutase 24, enolase 25, pyruvate kinase 26, pyruvate-NADP⁺ oxidoreductase (or pyruvate-ferredoxin oxidoreductase) 27, thiolase 28, 3-hydroxybutyryl-CoA dehydrogenase 29, crotonase 30, butyryl-CoA dehydrogenase 31, butyraldehyde dehydrogenase 32, and butanol dehydrogenase 33, with aldolase 20 and triose phosphate isomerase 21 being the only two additional enzymes relative to the glyceraldehydes-3-phosphate-branched designer pathway. The use of a pyruvate-NADP⁺ oxidoreductase 27 (instead of pyruvate-ferredoxin oxidoreductase) in catalyzing the conversion of a pyruvate molecule to acetyl-CoA enables production of an NADPH, which can be used in some other steps of the butanol-production pathway. The addition of yet one more enzyme in the designer organism, phosphofructose kinase 19, permits the creation of another designer pathway which branches off from the point of fructose-6-phosphate of the Calvin cycle for the production of butanol. Like the glyceraldehyde-3-phosphate-branched butanol-production pathway, both the fructose-1,6-diphosphate-branched pathway (20-33) and the fructose-6-phosphate-branched pathway (19-33) can themselves generate NADH for use in the pathway at the step catalyzed by 3-hydroxybutyryl-CoA dehydrogenase 29 to reduce acetoacetyl-CoA to 3-hydroxybutyryl-CoA and at the step catalyzed by butyryl-CoA dehydrogenase 31 to reduce crotonyl-CoA to butyryl-CoA. In each of these designer butanol-production pathways, the numbers of NADH molecules consumed are balanced with the numbers of NADH molecules generated; and both the butyraldehyde dehydrogenase 32 (catalyzing the step in reducing butyryl-CoA to butyraldehyde) and the butanol dehydrogenase 33 (catalyzing the terminal step in reducing butyraldehyde to butanol)

can all use NADPH, which can be regenerated by the photosynthetic water splitting and proton gradient-coupled electron transport process. Therefore, these designer butanol-production pathways can operate continuously.

[0063] Table 1 lists examples of the enzymes including those identified above for construction of the designer butanol-production pathways. Throughout this specification, when reference is made to an enzyme, such as, for example, any of the enzymes listed in Table 1, it includes their isozymes, functional analogs, and designer modified enzymes and combinations thereof. These enzymes can be selected for use in construction of the designer butanol-production pathways (such as those illustrated in FIG. 1). The “isozymes or functional analogs” refer to certain enzymes that have the same catalytic function but may or may not have exactly the same protein structures. The most essential feature of an enzyme is its active site that catalyzes the enzymatic reaction. Therefore, certain enzyme-protein fragment(s) or subunit(s) that contains such an active catalytic site may also be selected for use in this invention. For various reasons, some of the natural enzymes contain not only the essential catalytic structure but also other structure components that may or may not be desirable for a given application. With techniques of bioinformatics-assisted molecular designing, it is possible to select the essential catalytic structure(s) for use in construction of a designer DNA construct encoding a desirable designer enzyme. Therefore, in one of the various embodiments, a designer enzyme gene is created by artificial synthesis of a DNA construct according to bioinformatics-assisted molecular sequence design. With the computer-assisted synthetic biology approach, any DNA sequence (thus its protein structure) of a designer enzyme may be selectively modified to achieve more desirable results by design. Therefore, the terms “designer modified sequences” and “designer modified enzymes” are hereby defined as the DNA sequences and the enzyme proteins that are modified with bioinformatics-assisted molecular design. For example, when a DNA construct for a designer chloroplast-targeted enzyme is designed from the sequence of a mitochondrial enzyme, it is a preferred practice to modify some of the protein structures, for example, by selectively cutting out certain structure component(s) such as its mitochondrial transit-peptide sequence that is not suitable for the given application, and/or by adding certain peptide structures such as an exogenous chloroplast transit-peptide sequence (e.g., a 135-bp Rubisco small-subunit transit peptide (RbcS2)) that is needed to confer the ability in the chloroplast-targeted insertion of the designer protein. Therefore, one of the various embodiments flexibly employs the enzymes, their isozymes, functional analogs, designer modified enzymes, and/or the combinations thereof in construction of the designer butanol-production pathway (s).

[0064] As shown in Table 1, many genes of the enzymes identified above have been cloned and/or sequenced from various organisms. Both genomic DNA and/or mRNA sequence data can be used in designing and synthesizing the designer DNA constructs for transformation of a host alga, oxyphotobacterium, plant, plant tissue or cells to create a designer organism for photobiological butanol production (FIG. 1). However, because of possible variations often associated with various source organisms and cellular compartments with respect to a specific host organism and its chloroplast/thylakoid environment where the butanol-production pathway(s) is designed to work with the Calvin cycle, certain

molecular engineering art work in DNA construct design including codon-usage optimization and sequence modification is often necessary for a designer DNA construct (FIG. 2) to work well. For example, in creating a butanol-producing designer eukaryotic alga, if the source sequences are from cytosolic enzymes (sequences), a functional chloroplast-targeting sequence may be added to provide the capability for a designer nuclear gene-encoded enzyme to insert into a host chloroplast to confer its function for a designer butanol-production pathway. Furthermore, to provide the switchability for a designer butanol-production pathway, it is also important to include a functional inducible promoter sequence such as the promoter of a hydrogenase (Hyd1) or nitrate reductase (Nia1) gene, or nitrite reductase (nirA) gene in certain designer DNA construct(s) as illustrated in FIG. 2A to control the expression of designer gene(s). In addition, as mentioned before, certain functional derivatives or fragments of these enzymes (sequences), chloroplast-targeting transit peptide sequences, and inducible promoter sequences can also be selected for use in full, in part or in combinations thereof, to create the designer organisms according to various embodiments of this invention. The arts in creating and using the designer organisms are further described hereinbelow.

Targeting the Designer Enzymes to the Stroma Region of Chloroplasts

[0065] Some of the designer enzymes discussed above, such as, pyruvate-ferredoxin oxidoreductase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase are known to function in certain special bacteria such as *Clostridium*; but wild-type plant chloroplasts generally do not possess these enzymes to function with the Calvin cycle. Therefore, in one of the various embodiments in creating a butanol-producing eukaryotic designer organism, designer nucleic acids encoding for these enzymes are expressed in the chloroplast(s) of a host cell. This can be accomplished by delivery of designer butanol-production-pathway gene(s) into the chloroplast genome of the eukaryotic host cell typically using a gene gun. In certain extent, the molecular genetics of chloroplasts are similar to that of cyanobacteria. After being delivered into the chloroplast, a designer DNA construct that contains a pair of proper recombination sites as illustrated in FIG. 2F can be incorporated into the chloroplast genome through a natural process of homologous DNA double recombination.

[0066] In another embodiment, nucleic acids encoding for these enzymes are genetically engineered such that the enzymes expressed are inserted into the chloroplasts to operate with the Calvin cycle there. Depending on the genetic background of a particular host organism, some of the designer enzymes discussed above such as phosphoglycerate mutase and enolase may exist at some background levels in its native form in a wild-type chloroplast. For various reasons including often the lack of their controllability, however, some of the chloroplast background enzymes may or may not be sufficient to serve as a significant part of the designer butanol-production pathway(s). Furthermore, a number of useful inducible promoters happen to function in the nuclear genome. For example, both the hydrogenase (Hyd1) promoter and the nitrate reductase (Nia1) promoter that can be used to control the expression of the designer butanol-production pathways are located in the nuclear genome of *Chlamydomonas reinhardtii*, of which the genome has

recently been sequenced. Therefore, in one of the various embodiments, it is preferred to use nuclear-genome-encodable designer genes to confer a switchable butanol-production pathway. Consequently, nucleic acids encoding for these enzymes also need to be genetically engineered with proper sequence modification such that the enzymes are controllably expressed and are inserted into the chloroplasts to create a designer butanol-production pathway.

[0067] According to one of the various embodiments, it is best to express the designer butanol-producing-pathway enzymes only into chloroplasts (at the stroma region), exactly where the action of the enzymes is needed to enable photosynthetic production of butanol. If expressed without a chloroplast-targeted insertion mechanism, the enzymes would just stay in the cytosol and not be able to directly interact with the Calvin cycle for butanol production. Therefore, in addition to the obvious distinctive features in pathway designs and associated approaches, another significant distinction is that one of the various embodiments innovatively employs a chloroplast-targeted mechanism for genetic insertion of many designer butanol-production-pathway enzymes into chloroplast to directly interact with the Calvin cycle for photobiological butanol production.

[0068] With a chloroplast stroma-targeted mechanism, the cells will not only be able to produce butanol but also to grow and regenerate themselves when they are returned to certain conditions under which the designer pathway is turned off, such as under aerobic conditions when designer hydrogenase promoter-controlled butanol-production-pathway genes are used. Designer algae, plants, or plant cells that contain normal mitochondria should be able to use the reducing power (NADH) from organic reserves (and/or some exogenous organic substrate such as acetate or sugar) to power the cells immediately after returning to aerobic conditions. Consequently, when the designer algae, plants, or plant cells are returned to aerobic conditions after use under anaerobic conditions for photosynthetic butanol production, the cells will stop making the butanol-producing-pathway enzymes and start to restore the normal photoautotrophic capability by synthesizing new and functional chloroplasts. Therefore, it is possible to use such genetically engineered designer alga/plant organisms for repeated cycles of photoautotrophic growth under normal aerobic conditions and efficient production of butanol directly from CO₂ and H₂O under certain specific designer butanol-producing conditions such as under anaerobic conditions and/or in the presence of nitrate when a Nia1 promoter-controlled butanol-production pathway is used.

[0069] The targeted insertion of designer butanol-production-pathway enzymes can be accomplished through use of a DNA sequence that encodes for a stroma "signal" peptide. A stroma-protein signal (transit) peptide directs the transport and insertion of a newly synthesized protein into stroma. In accordance with one of the various embodiments, a specific targeting DNA sequence is preferably placed in between the promoter and a designer butanol-production-pathway enzyme sequence, as shown in a designer DNA construct (FIG. 2A). This targeting sequence encodes for a signal (transit) peptide that is synthesized as part of the apoprotein of an enzyme in the cytosol. The transit peptide guides the insertion of an apoprotein of a designer butanol-production-pathway enzyme from cytosol into the chloroplast. After the apopro-

tein is inserted into the chloroplast, the transit peptide is cleaved off from the apoprotein, which then becomes an active enzyme.

[0070] A number of transit peptide sequences are suitable for use for the targeted insertion of the designer butanol-production-pathway enzymes into chloroplast, including but not limited to the transit peptide sequences of: the hydrogenase apoproteins (such as HydA1 (Hyd1) and HydA2, GenBank accession number AJ308413, AF289201, AY090770), ferredoxin apoprotein (Frx1, accession numbers L10349, P07839), thioredoxin m apoprotein (Trx2, X62335), glutamine synthase apoprotein (Gs2, Q42689), LhcII apoproteins (AB051210, AB051208, AB051205), PSII-T apoprotein (PsbT), PSII-S apoprotein (PsbS), PSII-W apoprotein (PsbW), CF₀CF₁ subunit- δ apoprotein (AtpC), CF₀CF₁ subunit-6 apoprotein (AtpD, U41442), CF₀CF₁ subunit-II apoprotein (AtpG), photosystem I (PSI) apoproteins (such as, of genes PsaD, PsaE, PsaF, PsaG, PsaH, and PsaK), Rubisco SSU apoproteins (such as RbcS2, X04472). Throughout this specification, when reference is made to a transit peptide sequence, such as, for example, any of the transit peptide sequence described above, it includes their functional analogs, modified designer sequences, and combinations thereof. A “functional analog” or “modified designer sequence” in this context refers to a peptide sequence derived or modified (by, e.g., conservative substitution, moderate deletion or addition of amino acids, or modification of side chains of amino acids) based on a native transit peptide sequence, such as those identified above, that has the same function as the native transit peptide sequence, i.e., effecting targeted insertion of a desired enzyme.

[0071] In certain specific embodiments, the following transit peptide sequences are used to guide the insertion of the designer butanol-production-pathway enzymes into the stroma region of the chloroplast: the Hyd1 transit peptide (having the amino acid sequence: msalvlkpcavsvrgsscrarqvraprpl aastvrvala tleaparrlg nvaca (SEQ ID NO: 54)), the RbcS2 transit peptides (having the amino acid sequence: maaviakssv saavarpars svrpmaalkp avkaapvaap aqanq (SEQ ID NO: 55)), ferredoxin transit peptide (having the amino acid sequence: mamamrs (SEQ ID NO: 56)), the CF₀CF₁ subunit- δ transit peptide (having the amino acid sequence: mlaaksiagp rafkasavra apkagrrtvv vma (SEQ ID NO: 57)), their analogs, functional derivatives, designer sequences, and combinations thereof.

Use of a Genetic Switch to Control the Expression of a Designer Butanol-Producing Pathway.

[0072] Another key feature of the invention is the application of a genetic switch to control the expression of the designer butanol-producing pathway(s), as illustrated in FIG. 1. This switchability is accomplished through the use of an externally inducible promoter so that the designer transgenes are inducibly expressed under certain specific inducing conditions. Preferably, the promoter employed to control the expression of designer genes in a host is originated from the host itself or a closely related organism. The activities and inducibility of a promoter in a host cell can be tested by placing the promoter in front of a reporting gene, introducing this reporter construct into the host tissue or cells by any of the known DNA delivery techniques, and assessing the expression of the reporter gene.

[0073] In a preferred embodiment, the inducible promoter used to control the expression of designer genes is a promoter

that is inducible by anaerobiosis, i.e., active under anaerobic conditions but inactive under aerobic conditions. A designer alga/plant organism can perform autotrophic photosynthesis using CO₂ as the carbon source under aerobic conditions, and when the designer organism culture is grown and ready for photosynthetic butanol production, anaerobic conditions will be applied to turn on the promoter and the designer genes that encode a designer butanol-production pathway(s).

[0074] A number of promoters that become active under anaerobic conditions are suitable for use in the present invention. For example, the promoters of the hydrogenase genes (HydA1 (Hyd1) and HydA2, GenBank accession number: AJ308413, AF289201, AY090770) of *Chlamydomonas reinhardtii*, which is active under anaerobic conditions but inactive under aerobic conditions, can be used as an effective genetic switch to control the expression of the designer genes in a host alga, such as *Chlamydomonas reinhardtii*. In fact, *Chlamydomonas* cells contain several nuclear genes that are coordinately induced under anaerobic conditions. These include the hydrogenase structural gene itself (Hyd1), the Cyc6 gene encoding the apoprotein of Cytochrome C₆, and the Cpx1 gene encoding coprogen oxidase. The regulatory regions for the latter two have been well characterized, and a region of about 100 bp proves sufficient to confer regulation by anaerobiosis in synthetic gene constructs (Quinn, Barraco, Ericksson and Merchant (2000). “Coordinate copper- and oxygen-responsive Cyc6 and Cpx1 expression in *Chlamydomonas* is mediated by the same element.” *J Biol Chem* 275: 6080-6089). Although the above inducible algal promoters may be suitable for use in other plant hosts, especially in plants closely related to algae, the promoters of the homologous genes from these other plants, including higher plants, can be obtained and employed to control the expression of designer genes in those plants.

[0075] In another embodiment, the inducible promoter used in the present invention is an algal nitrate reductase (Nia1) promoter, which is inducible by growth in a medium containing nitrate and repressed in a nitrate-deficient but ammonium-containing medium (Loppes and Radoux (2002) “Two short regions of the promoter are essential for activation and repression of the nitrate reductase gene in *Chlamydomonas reinhardtii*,” *Mol Genet Genomics* 268: 42-48). Therefore, the Nia1 (gene accession number AF203033) promoter can be selected for use to control the expression of the designer genes in an alga according to the concentration levels of nitrate and ammonium in a culture medium. Additional inducible promoters that can also be selected for use in the present invention include, for example, the heat-shock protein promoter HSP70A (accession number: DQ059999, AY456093, M98823; Schroda, Blocker, Beek (2000) The HSP70A promoter as a tool for the improved expression of transgenes in *Chlamydomonas*. *Plant Journal* 21:121-131), the promoter of CabII-1 gene (accession number M24072), the promoter of Cal gene (accession number P20507), and the promoter of Ca2 gene (accession number P24258).

[0076] In the case of blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria), there are also a number of inducible promoters that can be selected for use in the present invention. For example, the promoters of the anaerobic-responsive bidirectional hydrogenase *hox* genes of *Nostoc* sp. PCC 7120 (GenBank: BA000019), *Prochlorothrix hollandica* (GenBank: U88400; *hoxUYH* operon promoter), *Synechocystis* sp. strain PCC 6803 (CyanoBase: sll1220 and sll1223), *Synechococcus elongatus*

PCC 6301 (CyanoBase: syc1235_c), *Arthrospira platensis* (GenBank: ABC26906), *Cyanothece* sp. CCY0110 (GenBank: ZP_01727419) and *Synechococcus* sp. PCC 7002 (GenBank: AAN03566), which are active under anaerobic conditions but inactive under aerobic conditions (Sjoholm, Oliveira, and Lindblad (2007) "Transcription and regulation of the bidirectional hydrogenase in the Cyanobacterium *Nostoc* sp. strain PCC 7120," *Applied and Environmental Microbiology*, 73(17): 5435-5446), can be used as an effective genetic switch to control the expression of the designer genes in a host oxyphotobacterium, such as *Nostoc* sp. PCC 7120, *Synechocystis* sp. strain PCC 6803, *Synechococcus elongatus* PCC 6301, *Cyanothece* sp. CCY0110, *Arthrospira platensis*, or *Synechococcus* sp. PCC 7002.

[0077] In another embodiment in creating switchable butanol-production designer organisms such as switchable designer oxyphotobacteria, the inducible promoter selected for use is a nitrite reductase (*nirA*) promoter, which is inducible by growth in a medium containing nitrate and repressed in a nitrate-deficient but ammonium-containing medium (Qi, Hao, Ng, Slater, Baszis, Weiss, and Valentin (2005) "Application of the *Synechococcus nirA* promoter to establish an inducible expression system for engineering the *Synechocystis* tocopherol pathway," *Applied and Environmental Microbiology*, 71(10): 5678-5684; Maeda, Kawaguchi, Ohe, and Omata (1998) "cis-Acting sequences required for NtcB-dependent, nitrite-responsive positive regulation of the nitrate assimilation operon in the Cyanobacterium *Synechococcus* sp. strain PCC 7942," *Journal of Bacteriology*, 180(16):4080-4088). Therefore, the *nirA* promoter sequences can be selected for use to control the expression of the designer genes in a number of oxyphotobacteria according to the concentration levels of nitrate and ammonium in a culture medium. The *nirA* promoter sequences that can be selected and modified for use include (but not limited to) the *nirA* promoters of the following oxyphotobacteria: *Synechococcus elongatus* PCC 6301 (GenBank: AP008231, region 355890-255950), *Synechococcus* sp. (GenBank: X67680.1, D16303.1, D12723.1, and D00677), *Synechocystis* sp. PCC 6803 (GenBank: NP_442378, BA000022, AB001339, D63999-D64006, D90899-D90917), *Anabaena* sp. (GenBank: X99708.1), *Nostoc* sp. PCC 7120 (GenBank: BA000019.2 and AJ319648), *Plectonema boryanum* (GenBank: D31732.1), *Synechococcus elongatus* PCC 7942 (GenBank: P39661, CP000100.1), *Thermosynechococcus elongatus* BP-1 (GenBank: BAC08901, NP_682139), *Phormidium laminosum* (GenBank: CAA79655, Q51879), *Mastigocladus laminosus* (GenBank: ABD49353, ABD49351, ABD49349, ABD49347), *Anabaena variabilis* ATCC 29413 (GenBank: YP_325032), *Prochlorococcus marinus* str. MIT 9303 (GenBank: YP_001018981), *Synechococcus* sp. WH 8103 (GenBank: AAC17122), *Synechococcus* sp. WH 7805 (GenBank: ZP_01124915), and *Cyanothece* sp. CCY0110 (GenBank: ZP_01727861).

[0078] In yet another embodiment, an inducible promoter selected for use is the light- and heat-responsive chaperone gene *groE* promoter, which can be induced by heat and/or light [Kojima and Nakamoto (2007) "A novel light- and heat-responsive regulation of the *groE* transcription in the absence of HrcA or CIRCE in cyanobacteria," FEBS Letters 581: 1871-1880). A number of *groE* promoters such as the *groES* and *groEL* (chaperones) promoters are available for use as an inducible promoter in controlling the expression of the designer butanol-production-pathway enzymes. The *groE*

promoter sequences that can be selected and modified for use in one of the various embodiments include (but not limited to) the *groES* and/or *groEL* promoters of the following oxyphotobacteria: *Synechocystis* sp. (GenBank: D12677.1), *Synechococcus* sp. PCC 6803 (GenBank: BA000022.2), *Synechococcus elongatus* PCC 6301 (GenBank: AP008231.1), *Synechococcus* sp. (GenBank: M58751.1), *Synechococcus elongatus* PCC 7942 (GenBank: CP000100.1), *Nostoc* sp. PCC 7120 (GenBank: BA000019.2), *Anabaena variabilis* ATCC 29413 (GenBank: CP000117.1), *Anabaena* sp. L-31 (GenBank: AF324500); *Thermosynechococcus elongatus* BP-1 (CyanoBase: t110185, t110186), *Synechococcus vulcanus* (GenBank: D78139), *Oscillatoria* sp. NKBG091600 (GenBank: AF054630), *Prochlorococcus marinus* MIT9313 (GenBank: BX572099), *Prochlorococcus marinus* str. MIT 9303 (GenBank: CP000554), *Prochlorococcus marinus* str. MIT 9211 (GenBank: ZP_01006613), *Synechococcus* sp. WH8102 (GenBank: BX569690), *Synechococcus* sp. CC9605 (GenBank: CP000110), *Prochlorococcus marinus* subsp. *marinus* str. CCMP1375 (GenBank: AE017126), and *Prochlorococcus marinus* MED4 (GenBank: BX548174).

[0079] Additional inducible promoters that can also be selected for use in the present invention include: for example, the metal (zinc)-inducible *smt* promoter of *Synechococcus* PCC 7942 (Erbe, Adams, Taylor and Hall (1996) "Cyanobacteria carrying an *smt-lux* transcriptional fusion as biosensors for the detection of heavy metal cations," *Journal of Industrial Microbiology*, 17:80-83); the iron-responsive *idiA* promoter of *Synechococcus elongatus* PCC 7942 (Michel, Pistorius, and Golden (2001) "Unusual regulatory elements for iron deficiency induction of the *idiA* gene of *Synechococcus elongatus* PCC 7942" *Journal of Bacteriology*, 183(17): 5015-5024); the redox-responsive cyanobacterial *crhR* promoter (Patterson-Fortin, Colvin and Owtrim (2006) "A LexA-related protein regulates redox-sensitive expression of the cyanobacterial RNA helicase, *crhR*", *Nucleic Acids Research*, 34(12):3446-3454); the heat-shock gene *hsp16.6* promoter of *Synechocystis* sp. PCC 6803 (Fang and Barnum (2004) "Expression of the heat shock gene *hsp16.6* and promoter analysis in the Cyanobacterium, *Synechocystis* sp. PCC 6803," *Current Microbiology* 49:192-198); the small heat-shock protein (*Hsp*) promoter such as *Synechococcus vulcanus* gene *hspA* promoter (Nakamoto, Suzuki, and Roy (2000) "Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria," FEBS Letters 483:169-174); the CO₂-responsive promoters of oxyphotobacterial carbonic-anhydrase genes (GenBank: EAZ90903, EAZ90685, ZP_01624337, EAW33650, ABB17341, AAT41924, CAO89711, ZP_00111671, YP_400464, AAC44830; and CyanoBase: all2929, PMT1568 slr0051, slr1347, and syc0167_c); the nitrate-reductase-gene (*narB*) promoters (such as GenBank accession numbers: BAC08907, NP_682145, AAO25121; ABI46326, YP_732075, BAB72570, NP_484656); the green/red light-responsive promoters such as the light-regulated *cpcB2A2* promoter of *Fremyella diplosiphon* (Casey and Grossman (1994) "In vivo and in vitro characterization of the light-regulated *cpcB2A2* promoter of *Fremyella diplosiphon*" *Journal of Bacteriology*, 176(20):6362-6374); and the UV-light responsive promoters of cyanobacterial genes *lexA*, *recA* and *ruvB* (Domain, Houot, Chauvat, and Cassier-Chauvat (2004) "Function and regulation of the cyanobacterial

genes *lexA*, *recA* and *ruvB*: *LexA* is critical to the survival of cells facing inorganic carbon starvation," *Molecular Microbiology*, 53(1):65-80).

[0080] Furthermore, in one of the various embodiments, certain "semi-inducible" or constitutive promoters can also be selected for use in combination of an inducible promoter (s) for construction of a designer butanol-production pathway (s) as well. For example, the promoters of oxyphotobacterial Rubisco operon such as the *rbcL* genes (GenBank: X65960, ZP_01728542, Q3M674, BAF48766, NP_895035, 0907262A; CyanoBase: PMT1205, PMM0550, Pro0551, tll1506, SYNW1718, glr2156, alr1524, slr0009), which have certain light-dependence but could be regarded almost as constitutive promoters, can also be selected for use in combination of an inducible promoter(s) such as the *nirA*, *hox*, and/or *groE* promoters for construction of the designer butanol-production pathway(s) as well.

[0081] Throughout this specification, when reference is made to inducible promoter, such as, for example, any of the inducible promoters described above, it includes their analogs, functional derivatives, designer sequences, and combinations thereof. A "functional analog" or "modified designer sequence" in this context refers to a promoter sequence derived or modified (by, e.g., substitution, moderate deletion or addition or modification of nucleotides) based on a native promoter sequence, such as those identified hereinabove, that retains the function of the native promoter sequence.

DNA Constructs and Transformation into Host Organisms

[0082] DNA constructs are generated in order to introduce designer butanol-production-pathway genes to a host alga, plant, plant tissue or plant cells. That is, a nucleotide sequence encoding a designer butanol-production-pathway enzyme is placed in a vector, in an operable linkage to a promoter, preferably an inducible promoter, and in an operable linkage to a nucleotide sequence coding for an appropriate chloroplast-targeting transit-peptide sequence. In a preferred embodiment, nucleic acid constructs are made to have the elements placed in the following 5' (upstream) to 3' (downstream) orientation: an externally inducible promoter, a transit targeting sequence, and a nucleic acid encoding a designer butanol-production-pathway enzyme, and preferably an appropriate transcription termination sequence. One or more designer genes (DNA constructs) can be placed into one genetic vector. An example of such a construct is depicted in FIG. 2A. As shown in the embodiment illustrated in FIG. 2A, a designer butanol-production-pathway transgene is a nucleic acid construct comprising: a) a PCR forward primer; b) an externally inducible promoter; c) a transit targeting sequence; d) a designer butanol-production-pathway-enzyme-encoding sequence with an appropriate transcription termination sequence; and e) a PCR reverse primer.

[0083] In accordance with various embodiments, any of the components a) through e) of this DNA construct are adjusted to suit for certain specific conditions. In practice, any of the components a) through e) of this DNA construct are applied in full or in part, and/or in any adjusted combination to achieve more desirable results. For example, when an algal hydrogenase promoter is used as an inducible promoter in the designer butanol-production-pathway DNA construct, a transgenic designer alga that contains this DNA construct will be able to perform autotrophic photosynthesis using ambient-air CO₂ as the carbon source and grows normally under aerobic conditions, such as in an open pond. When the algal culture is grown and ready for butanol production, the

designer transgene(s) can then be expressed by induction under anaerobic conditions because of the use of the hydrogenase promoter. The expression of designer gene(s) produces a set of designer butanol-production-pathway enzymes to work with the Calvin cycle for photobiological butanol production (FIG. 1).

[0084] The two PCR primers are a PCR forward primer (PCR FD primer) located at the beginning (the 5' end) of the DNA construct and a PCR reverse primer (PCR RE primer) located at the other end (the 3' end) as shown in FIG. 2A. This pair of PCR primers is designed to provide certain convenience when needed for relatively easy PCR amplification of the designer DNA construct, which is helpful not only during and after the designer DNA construct is synthesized in preparation for gene transformation, but also after the designer DNA construct is delivered into the genome of a host alga for verification of the designer gene in the transformants. For example, after the transformation of the designer gene is accomplished in a *Chlamydomonas reinhardtii-arg7* host cell using the techniques of electroporation and argininosuccinate lyase (*arg7*) complementation screening, the resulted transformants can be then analyzed by a PCR DNA assay of their nuclear DNA using this pair of PCR primers to verify whether the entire designer butanol-production-pathway gene (the DNA construct) is successfully incorporated into the genome of a given transformant. When the nuclear DNA PCR assay of a transformant can generate a PCR product that matches with the predicted DNA size and sequence according to the designer DNA construct, the successful incorporation of the designer gene(s) into the genome of the transformant is verified.

[0085] Therefore, the various embodiments also teach the associated method to effectively create the designer transgenic algae, plants, or plant cells for photobiological butanol production. This method, in one of embodiments, includes the following steps: a) Selecting an appropriate host alga, plant, plant tissue, or plant cells with respect to their genetic backgrounds and special features in relation to butanol production; b) Introducing the nucleic acid constructs of the designer genes into the genome of said host alga, plant, plant tissue, or plant cells; c) Verifying the incorporation of the designer genes in the transformed alga, plant, plant tissue, or plant cells with DNA PCR assays using the said PCR primers of the designer DNA construct; d) Measuring and verifying the designer organism features such as the inducible expression of the designer butanol-pathway genes for photosynthetic butanol production from carbon dioxide and water by assays of mRNA, protein, and butanol-production characteristics according to the specific designer features of the DNA construct(s) (FIG. 2A).

[0086] The above embodiment of the method for creating the designer transgenic organism for photobiological butanol production can also be repeatedly applied for a plurality of operational cycles to achieve more desirable results. In various embodiments, any of the steps a) through d) of this method described above are adjusted to suit for certain specific conditions. In various embodiments, any of the steps a) through d) of the method are applied in full or in part, and/or in any adjusted combination.

[0087] Examples of designer butanol-production-pathway genes (DNA constructs) are shown in the sequence listings. SEQ ID NO: 1 presents a detailed DNA construct of a designer Butanol Dehydrogenase gene (1809 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate

reductase Nial promoter (21-282), a 135-bp RbcS2 transit peptide (283-417), an enzyme-encoding sequence (418-1566) selected and modified from a *Clostridium saccharoperbutylacetonicum* Butanol Dehydrogenase sequence (AB257439), a 223-bp RbcS2 terminator (1567-1789), and a PCR RE primer (1790-1809). The 262-bp Nial promoter (DNA sequence 21-282) is used as an example of an inducible promoter to control the expression of a designer butanol-production-pathway Butanol Dehydrogenase gene (DNA sequence 418-1566). The 135-bp RbcS2 transit peptide (DNA sequence 283-417) is used as an example to guide the insertion of the designer enzyme (DNA sequence 418-1566) into the chloroplast of the host organism. The RbcS2 terminator (DNA sequence 1567-1789) is employed so that the transcription and translation of the designer gene is properly terminated to produce the designer apoprotein (RbcS2 transit peptide-Butanol Dehydrogenase) as desired. Because the Nial promoter is a nuclear DNA that can control the expression only for nuclear genes, the synthetic butanol-production-pathway gene in this example is designed according to the codon usage of *Chlamydomonas* nuclear genome. Therefore, in this case, the designer enzyme gene is transcribed in nucleus. Its mRNA is naturally translocated into cytosol, where the mRNA is translated to an apoprotein that consists of the RbcS2 transit peptide (corresponding to DNA sequence 283-417) with its C-terminal end linked together with the N-terminal end of the Butanol Dehydrogenase protein (corresponding to DNA sequence 418-1566). The transit peptide of the apoprotein guides its transportation across the chloroplast membranes and into the stroma area, where the transit peptide is cut off from the apoprotein. The resulting Butanol Dehydrogenase then resumes its function as an enzyme for the designer butanol-production pathway in chloroplast. The two PCR primers (sequences 1-20 and 1790-1809) are selected and modified from the sequence of a Human actin gene and can be paired with each other. Blasting the sequences against *Chlamydomonas* GenBank found no homologous sequences of them. Therefore, they can be used as appropriate PCR primers in DNA PCR assays for verification of the designer gene in the transformed alga.

[0088] SEQ ID NO: 2 presents example 2 for a designer Butyraldehyde Dehydrogenase DNA construct (2067 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase Nial promoter (21-282), a 135-bp RbcS2 transit peptide (283-417), a Butyraldehyde Dehydrogenase-encoding sequence (418-1824) selected and modified from a *Clostridium saccharoperbutylacetonicum* Butyraldehyde Dehydrogenase sequence (AY251646), a 223-bp RbcS2 terminator (1825-2047), and a PCR RE primer (2048-2067). This DNA construct is similar to example 1, SEQ ID NO: 1, except that a Butyraldehyde Dehydrogenase-encoding sequence (418-1824) selected and modified from a *Clostridium saccharoperbutylacetonicum* Butyraldehyde Dehydrogenase sequence (AY251646) is used.

[0089] SEQ ID NO: 3 presents example 3 for a designer Butyryl-CoA Dehydrogenase construct (1815 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase promoter (21-282), a 9-bp Xho I NdeI site (283-291), a 135-bp RbcS2 transit peptide (292-426), a Butyryl-CoA Dehydrogenase encoding sequence (427-1563) selected/modified from the sequences of a *Clostridium beijerinckii* Butyryl-CoA Dehydrogenase (AF494018), a 9-bp XbaI site (1564-1572), a 223-bp RbcS2 terminator (1573-1795), and a PCR RE primer (1796-1815) at the 3' end.

This DNA construct is similar to example 1, SEQ ID NO: 1, except that a Butyryl-CoA Dehydrogenase encoding sequence (427-1563) selected/modified from the sequences of a *Clostridium beijerinckii* Butyryl-CoA Dehydrogenase (AF494018) is used and restriction sites of Xho I NdeI and XbaI are added to make the key components such as the targeting sequence (292-426) and the designer enzyme sequence (427-1563) as a modular unit that can be flexible replaced when necessary to save cost of gene synthesis and enhance work productivity. Please note, the enzyme does not have to be *Clostridium beijerinckii* Butyryl-CoA Dehydrogenase; a number of butyryl-CoA dehydrogenase enzymes (such as those listed in Table 1) including their isozymes, designer modified enzymes, and functional analogs from other sources such as *Butyrivibrio fibrisolvens*, Butyrate producing bacterium L2-50, *Thermoanaerobacterium thermo-saccharolyticum*, can also be selected for use.

[0090] SEQ ID NO: 4 presents example 4 for a designer Crotonase DNA construct (1482 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase promoter (21-282), a 9-bp Xho I NdeI site (283-291) a 135-bp RbcS2 transit peptide (292-426), a Crotonase-encoding sequence (427-1209) selected/modified from the sequences of a *Clostridium beijerinckii* Crotonase (Genbank: AF494018), a 21-bp Lumio-tag-encoding sequence (1210-1230), a 9-bp XbaI site (1231-1239) containing a stop codon, a 223-bp RbcS2 terminator (1240-1462), and a PCR RE primer (1463-1482) at the 3' end. This DNA construct is similar to example 3, SEQ ID NO: 3, except that a Crotonase-encoding sequence (427-1209) selected/modified from the sequences of a *Clostridium beijerinckii* Crotonase (Genbank: AF494018) is used and a 21-bp Lumio-tag-encoding sequence (1210-1230) is added at the C-terminal end of the enolase sequence. The 21-bp Lumio-tag sequence (1210-1230) is employed here to encode a Lumio peptide sequence Gly-Cys-Cys-Pro-Gly-Cys-Cys, which can become fluorescent when treated with a Lumio reagent that is now commercially available from Invitrogen [<https://catalog.invitrogen.com>]. Lumio molecular tagging technology is based on an EDT (1,2-ethanedithiol) coupled biarsenical derivative (the Lumio reagent) of fluorescein that binds to an engineered tetracysteine sequence (Keppepola, Coffman, and et al (2003). Rapid detection of in vitro expressed proteins using Lumio™ technology, *Gene Expression*, 25.3:7-11). The tetracysteine sequence consists of Cys-Cys-Xaa-Xaa-Cys-Cys, where Xaa is any non-cysteine amino acid such as Pro or Gly in this example. The EDT-linked Lumio reagent allows free rotation of the arsenic atoms that quenches the fluorescence of fluorescein. Covalent bond formation between the thiols of the Lumio's arsenic groups and the tetracysteines prevents free rotation of arsenic atoms that releases the fluorescence of fluorescein (Griffin, Adams, and Tsien (1998), "Specific covalent labeling of recombinant protein molecules inside live cells", *Science*, 281:269-272). This also permits the visualization of the tetracysteine-tagged proteins by fluorescent molecular imaging. Therefore, use of the Lumio tag in this manner enables monitoring and/or tracking of the designer Crotonase when expressed to verify whether the designer butanol-production pathway enzyme is indeed delivered into the chloroplast of a host organism as designed. The Lumio tag (a short 7 amino acid peptide) that is linked to the C-terminal end of the Crotonase protein in this example should have minimal effect on the function of the designer enzyme, but enable the designer enzyme molecule to be visualized when treated with the

Lumio reagent. Use of the Lumio tag is entirely optional. If the Lumio tag somehow affects the designer enzyme function, this tag can be deleted in the DNA sequence design.

[0091] SEQ ID NO: 5 presents example 5 for a designer 3-Hydroxybutyryl-CoA Dehydrogenase DNA construct (1367 bp) that includes a PCR FD primer (sequence 1-20), a 84-bp nitrate reductase promoter (21-104), a 9-bp Xho I NdeI site (105-113) a 135-bp RbcS2 transit peptide (114-248), a 3-Hydroxybutyryl-CoA Dehydrogenase-encoding sequence (249-1094) selected/modified from a *Clostridium beijerinckii* 3-Hydroxybutyryl-CoA Dehydrogenase sequence (Genbank: AF494018), a 21-bp Lumio-tag sequence (1095-1115), a 9-bp XbaI site (1116-1124), a 223-bp RbcS2 terminator (1125-1347), and a PCR RE primer (1348-1367). This DNA construct is similar to example 4, SEQ ID NO: 4, except that an 84-bp nitrate reductase promoter (21-104) and a 3-Hydroxybutyryl-CoA Dehydrogenase-encoding sequence (249-1094) selected/modified from a *Clostridium beijerinckii* 3-Hydroxybutyryl-CoA Dehydrogenase sequence (Genbank: AF494018) are used. The 84-bp nitrate-reductase promoter is artificially created by joining two partially homologous sequence regions (-231 to -201 and -77 to -25 with respect to the start site of transcription) of the native *Chlamydomonas reinhardtii* Nia1 promoter. Experimental studies have demonstrated that the 84-bp sequence is more active than the native Nia1 promoter (Loppes and Radoux (2002) "Two short regions of the promoter are essential for activation and repression of the nitrate reductase gene in *Chlamydomonas reinhardtii*," *Mol Genet Genomics* 268: 42-48). Therefore, this is also an example where functional synthetic sequences, analogs, functional derivatives and/or designer modified sequences such as the synthetic 84-bp sequence can be selected for use according to various embodiments in this invention.

[0092] SEQ ID NO: 6 presents example 6 for a designer Thiolase DNA construct (1721 bp) that includes a PCR FD primer (sequence 1-20), a 84-bp nitrate reductase promoter (21-104), a 9-bp Xho I NdeI site (105-113) a 135-bp RbcS2 transit peptide (114-248), a Thiolase-encoding sequence (248-1448) selected/modified from a *Butyrivibrio fibrisolvens* Thiolase sequence (AB190764), a 21-bp Lumio-tag sequence (1449-1469), a 9-bp XbaI site (1470-1478), a 223-bp RbcS2 terminator (1479-1701), and a PCR RE primer (1702-1721). This DNA construct is also similar to example 4, SEQ ID NO: 4, except that a Thiolase-encoding-encoding sequence (249-1448) and an 84-bp synthetic Nia1 promoter (21-104) are used. This is another example that functional synthetic sequences can also be selected for use in designer DNA constructs.

[0093] SEQ ID NO: 7 presents example 7 for a designer Pyruvate-Ferredoxin Oxidoreductase DNA construct (4211 bp) that includes a PCR FD primer (sequence 1-20), a 2x84-bp nitrate reductase promoter (21-188), a 9-bp Xho I NdeI site (189-197) a 135-bp RbcS2 transit peptide (198-332), a Pyruvate-Ferredoxin Oxidoreductase-encoding sequence (333-3938) selected/modified from the sequences of a *Mastigamoeba balamuthi* Pyruvate-ferredoxin oxidoreductase (GenBank: AY101767), a 21-bp Lumio-tag sequence (3939-3959), a 9-bp XbaI site (3960-3968), a 223-bp RbcS2 terminator (3969-4191), and a PCR RE primer (4192-4211). This DNA construct is also similar to example 4, SEQ ID NO: 4, except a designer 2x84-bp Nia1 promoter and a Pyruvate-Ferredoxin Oxidoreductase-encoding sequence (333-3938) selected/modified from the sequences of a *Mastigamoeba*

balamuthi Pyruvate-ferredoxin oxidoreductase (GenBank: AY101767) are used. The 2x84-bp Nia1 promoter is constructed as a tandem duplication of the 84-bp synthetic Nia1 promoter sequence presented in SEQ ID NO: 6 above. Experimental tests have shown that the 2x84-bp synthetic Nia1 promoter is even more powerful than the 84-bp sequence which is more active than the native Nia1 promoter (Loppes and Radoux (2002) "Two short regions of the promoter are essential for activation and repression of the nitrate reductase gene in *Chlamydomonas reinhardtii*," *Mol Genet Genomics* 268: 42-48). Use of this type of inducible promoter sequences with various promoter strengths can also help in adjusting the expression levels of the designer enzymes for the butanol-production pathway(s).

[0094] SEQ ID NO: 8 presents example 8 for a designer Pyruvate Kinase DNA construct (2021 bp) that includes a PCR FD primer (sequence 1-20), a 84-bp nitrate reductase promoter (21-104), a 9-bp Xho I NdeI site (105-113) a 135-bp RbcS2 transit peptide (114-248), a pyruvate kinase-encoding sequence (249-1748) selected/modified from a *Saccharomyces cerevisiae* Pyruvate Kinase sequence (GenBank: AY949876), a 21-bp Lumio-tag sequence (1749-1769), a 9-bp XbaI site (1770-1778), a 223-bp RbcS2 terminator (1779-2001), and a PCR RE primer (2002-2021). This DNA construct is similar to example 6, SEQ ID NO: 6, except that a pyruvate kinase-encoding sequence (249-1748) is used.

[0095] SEQ ID NO: 9 presents example 9 for a designer Enolase gene (1815 bp) consisting of a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase promoter (21-282), a 9-bp Xho I NdeI site (283-291) a 135-bp RbcS2 transit peptide (292-426), a enolase-encoding sequence (427-1542) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic enolase (Genbank: X66412, P31683), a 21-bp Lumio-tag-encoding sequence (1507-1527), a 9-bp XbaI site (1543-1551) containing a stop codon, a 223-bp RbcS2 terminator (1552-1795), and a PCR RE primer (1796-1815) at the 3' end. This DNA construct is similar to example 3, SEQ ID NO: 3, except that an enolase-encoding sequence (427-1542) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic enolase is used.

[0096] SEQ ID NO: 10 presents example 10 for a designer Phosphoglycerate-Mutase DNA construct (2349 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase promoter (21-282), a 9-bp Xho I NdeI site (283-291), a 135-bp RbcS2 transit peptide (292-426), a phosphoglycerate-mutase encoding sequence (427-2097) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic phosphoglycerate mutase (JGI Chlre2 protein ID 161689, Genbank: AF268078), a 9-bp XbaI site (2098-2106), a 223-bp RbcS2 terminator (2107-2329), and a PCR RE primer (2330-2349) at the 3' end. This DNA construct is similar to example 3, SEQ ID NO: 3, except that a phosphoglycerate-mutase encoding sequence (427-2097) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic phosphoglycerate mutase is used.

[0097] SEQ ID NO: 11 presents example 11 for a designer Phosphoglycerate Kinase DNA construct (1908 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase Nia1 promoter (21-282), a phosphoglycerate-kinase-encoding sequence (283-1665) selected from a *Chlamydomonas reinhardtii* chloroplast phosphoglycerate-kinase sequence including its chloroplast signal peptide and mature enzyme sequence (GenBank: U14912), a 223-bp RbcS2 terminator (1666-1888), and a PCR RE primer (1889-1908).

This DNA construct is similar to example 1, SEQ ID NO: 1, except a phosphoglycerate-kinase-encoding sequence (283-1665) selected from a *Chlamydomonas reinhardtii* chloroplast phosphoglycerate-kinase sequence including its chloroplast signal peptide and mature enzyme sequence is used. Therefore, this is also an example where the sequence of a nuclear-encoded chloroplast enzyme such as the *Chlamydomonas reinhardtii* chloroplast phosphoglycerate kinase can also be used in design and construction of a designer butanol-production pathway gene when appropriate with a proper inducible promoter such as the Nial1 promoter (DNA sequence 21-282).

[0098] SEQ ID NO: 12 presents example 12 for a designer Glyceraldehyde-3-Phosphate Dehydrogenase gene (1677 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase Nial1 promoter (21-282), a 135-bp RbcS2 transit peptide (283-417), an enzyme-encoding sequence (418-1434) selected and modified from a *Mesostigma viride* cytosolic glyceraldehyde-3-phosphate dehydrogenase (mRNA) sequence (GenBank accession number DQ873404), a 223-bp RbcS2 terminator (1435-1657), and a PCR RE primer (1658-1677). This DNA construct is similar to example 1, SEQ ID NO: 1, except that an enzyme-encoding sequence (418-1434) selected and modified from a *Mesostigma viride* cytosolic glyceraldehyde-3-phosphate dehydrogenase (mRNA) sequence (GenBank accession number DQ873404) is used.

[0099] SEQ ID NO: 13 presents example 13 for a designer HydA1-promoter-linked Phosphoglycerate Mutase DNA construct (2351 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a phosphoglycerate-mutase encoding sequence (438-2108) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic phosphoglycerate mutase (JGI Chlr2 protein ID 161689, GenBank: AF268078), a 223-bp RbcS2 terminator (2109-2331), and a PCR RE primer (2332-2351). This designer DNA construct is quite similar to example 1, SEQ ID NO:1, except that a 282-bp HydA1 promoter (21-302) and a phosphoglycerate-mutase encoding sequence (438-2108) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic phosphoglycerate mutase are used. The 282-bp HydA1 promoter (21-302) has been proven active by experimental assays at the inventor's laboratory. Use of the HydA1 promoter (21-302) enables activation of designer enzyme expression by using anaerobic culture-medium conditions.

[0100] With the same principle of using an inducible anaerobic promoter and a chloroplast-targeting sequence as that shown in SEQ ID NO: 13 (example 13), SEQ ID NOS: 14-23 show designer-gene examples 14-23. Briefly, SEQ ID NO: 14 presents example 14 for a designer HydA1-promoter-linked Enolase DNA construct (1796 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Enolase-encoding sequence (438-1553) selected/modified from the sequences of a *Chlamydomonas reinhardtii* cytosolic enolase (GenBank: X66412, P31683), a 223-bp RbcS2 terminator (1554-1776), and a PCR RE primer (1777-1796).

[0101] SEQ ID NO: 15 presents example 15 for a designer HydA1-promoter-controlled Pyruvate-Kinase DNA construct that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Pyruvate Kinase-encoding sequence (438-1589) selected/modified from a *Chlamydomonas rein-*

hardtii cytosolic pyruvate kinase sequence (JGI Chlr3 protein ID 138105), a 223-bp RbcS2 terminator (1590-1812), and a PCR RE primer (1813-1832).

[0102] SEQ ID NO:16 presents example 16 for a designer HydA1-promoter-linked Pyruvate-ferredoxin oxidoreductase DNA construct (4376 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Pyruvate-ferredoxin oxidoreductase-encoding sequence (438-4133) selected/modified from a *Desulfovibrio africanus* Pyruvate-ferredoxin oxidoreductase sequence (GenBank Accession Number Y09702), a 223-bp RbcS2 terminator (4134-4356), and a PCR RE primer (4357-4376).

[0103] SEQ ID NO:17 presents example 17 for a designer HydA1-promoter-linked Pyruvate-NADP⁺ oxidoreductase DNA construct (6092 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Pyruvate-NADP⁺ oxidoreductase-encoding sequence (438-5849) selected/modified from a *Euglena gracilis* Pyruvate-NADP⁺ oxidoreductase sequence (GenBank Accession Number AB021127), a 223-bp RbcS2 terminator (5850-6072), and a PCR RE primer (6073-6092).

[0104] SEQ ID NO:18 presents example 18 for a designer HydA1-promoter-linked Thiolase DNA construct (1856 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Thiolase-encoding sequence (438-1613) selected/modified from the sequences of a *Thermoanaerobacterium thermosaccharolyticum* Thiolase (GenBank Z92974), a 223-bp RbcS2 terminator (1614-1836), and a PCR RE primer (1837-1856).

[0105] SEQ ID NO:19 presents example 19 for a designer HydA1-promoter-linked 3-Hydroxybutyryl-CoA dehydrogenase DNA construct (1550 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a 3-Hydroxybutyryl-CoA dehydrogenase-encoding sequence (438-1307) selected/modified from the sequences of a *Thermoanaerobacterium thermosaccharolyticum* 3-Hydroxybutyryl-CoA dehydrogenase (GenBank Z92974), a 223-bp RbcS2 terminator (1308-1530), and a PCR RE primer (1531-1550).

[0106] SEQ ID NO:20 presents example 20 for a designer HydA1-promoter-linked Crotonase DNA construct (1457 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Crotonase-encoding sequence (438-1214) selected/modified from the sequences of a *Thermoanaerobacterium thermosaccharolyticum* Crotonase (GenBank Z92974), a 223-bp RbcS2 terminator (1215-1437), and a PCR RE primer (1438-1457).

[0107] SEQ ID NO:21 presents example 21 for a designer HydA1-promoter-linked Butyryl-CoA dehydrogenase DNA construct (1817 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Butyryl-CoA dehydrogenase-encoding sequence (438-1574) selected/modified from the sequences of a *Thermoanaerobacterium thermosaccharolyticum* Butyryl-CoA dehydrogenase (GenBank Z92974), a 223-bp RbcS2 terminator (1575-1797), and a PCR RE primer (1798-1817).

[0108] SEQ ID NO: 22 presents example 22 for a designer HydA1-promoter-linked Butyraldehyde dehydrogenase DNA construct (2084 bp) that includes a PCR FD primer

(sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Butyraldehyde dehydrogenase-encoding sequence (438-1841) selected/modified from the sequences of a *Clostridium saccharoperbutylacetonicum* Butyraldehyde dehydrogenase (GenBank AY251646), a 223-bp RbcS2 terminator (1842-2064), and a PCR RE primer (2065-2084).

[0109] SEQ ID NO: 23 presents example 23 for a designer HydA1-promoter-linked Butanol dehydrogenase DNA construct (1733 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a 135-bp RbcS2 transit peptide (303-437), a Butanol dehydrogenase-encoding sequence (438-1490) selected/modified from the sequences of a *Clostridium beijerinckii* Butanol dehydrogenase (GenBank AF157307), a 223-bp RbcS2 terminator (1491-1713), and a PCR RE primer (1714-1733).

[0110] With the same principle of using a 2x84 synthetic Nial promoter and a chloroplast-targeting mechanism as mentioned previously, SEQ ID NOS:24-26 show more examples of designer-enzyme DNA-constructs. Briefly, SEQ ID NO: 24 presents example 24 for a designer Fructose-Diphosphate-Aldolase DNA construct that includes a PCR FD primer (sequence 1-20), a 2x84-bp NR promoter (21-188), a Fructose-Diphosphate Aldolase-encoding sequence (189-1313) selected/modified from a *C. reinhardtii* chloroplast fructose-1,6-bisphosphate aldolase sequence (GenBank: X69969), a 223-bpRbcS2 terminator (1314-1536), and a PCR RE primer (1537-1556).

[0111] SEQ ID NO: 25 presents example 24 for a designer Triose-Phosphate-Isomerase DNA construct that includes a PCR FD primer (sequence 1-20), a 2x84-bp NR promoter (21-188), a Triose-Phosphate Isomerase-encoding sequence (189-1136) selected and modified from a *Arabidopsis thaliana* chloroplast triosephosphate-isomerase sequence (GenBank: AF247559), a 223-bp RbcS2 terminator (1137-1359), and a PCR RE primer (1360-1379).

[0112] SEQ ID NO: 26 presents example 26 for a designer Phosphofructose-Kinase DNA construct that includes a PCR FD primer (sequence 1-20), a 2x84-bp NR promoter (21-188), a 135-bp RbcS2 transit peptide (189-323), a Phosphofructose Kinase-encoding sequence (324-1913) selected/modified from *Arabidopsis thaliana* 6-phosphofructokinase sequence (GenBank: NM_001037043), a 223-bp RbcS2 terminator (1914-2136), and a PCR RE primer (2137-2156).

[0113] The nucleic acid constructs, such as those presented in the examples above, may include additional appropriate sequences, for example, a selection marker gene, and an optional biomolecular tag sequence (such as the Lumio tag described in example 4, SEQ ID NO: 4). Selectable markers that can be selected for use in the constructs include markers conferring resistances to kanamycin, hygromycin, spectinomycin, streptomycin, sulfonyl urea, gentamycin, chloramphenicol, among others, all of which have been cloned and are available to those skilled in the art. Alternatively, the selective marker is a nutrition marker gene that can complement a deficiency in the host organism. For example, the gene encoding argininosuccinate lyase (*arg7*) can be used as a selection marker gene in the designer construct, which permits identification of transformants when *Chlamydomonas reinhardtii arg7*-(minus) cells are used as host cells.

[0114] Nucleic acid constructs carrying designer genes can be delivered into a host alga, blue-green alga, plant, or plant tissue or cells using the available gene-transformation techniques, such as electroporation, PEG induced uptake, and

ballistic delivery of DNA, and *Agrobacterium*-mediated transformation. For the purpose of delivering a designer construct into algal cells, the techniques of electroporation, glass bead, and biolistic gene gun can be selected for use as preferred methods; and an alga with single cells or simple thallus structure is preferred for use in transformation. Transformants can be identified and tested based on routine techniques.

[0115] The various designer genes can be introduced into host cells sequentially in a step-wise manner, or simultaneously using one construct or in one transformation. For example, the ten DNA constructs shown in SEQ ID NO: 13-16 (or 17) and 18-23 for the ten-enzyme 3-phosphoglycerate-branched butanol-production pathway can be placed into a genetic vector such as p389-Arg7 with a single selection marker (*Arg7*). Therefore, by use of a plasmid in this manner, it is possible to deliver all the ten DNA constructs (designer genes) into an arginine-requiring *Chlamydomonas reinhardtii-arg7* host (CC-48) in one transformation for expression of the 3-phosphoglycerate-branched butanol-production pathway (03-12 in FIG. 1). When necessary, a transformant containing the ten DNA constructs can be further transformed to get more designer genes into its genomic DNA with an additional selection marker such as streptomycin. By using combinations of various designer-enzymes DNA constructs such as those presented in SEQ ID NO: 1-26 in genetic transformation with an appropriate host organism, various butanol-production pathways such as those illustrated in FIG. 1 can be constructed. For example, the designer DNA constructs of SEQ ID NO: 1-12 can be selected for construction of the glyceraldehydes-3-phosphate-branched butanol-production pathway (01-12 in FIG. 1); The designer DNA constructs of SEQ ID NO: 1-12, 24, and 25 can be selected for construction of the fructose-1,6-diphosphate-branched butanol-production pathway (20-33); and the designer DNA constructs of SEQ ID NO: 1-12 and 24-26 can be selected for construction of the fructose-6-phosphate-branched butanol-production pathway (19-33).

Additional Host Modifications to Enhance Photosynthetic Butanol Production

An NADPH/NADH Conversion Mechanism

[0116] According to the photosynthetic butanol production pathway(s), to produce one molecule of butanol from 4CO₂ and 5H₂O is likely to require 14 ATP and 12 NADPH, both of which are generated by photosynthetic water splitting and photophosphorylation across the thylakoid membrane. In order for the 3-phosphoglycerate-branched butanol-production pathway (03-12 in FIG. 1) to operate, it is a preferred practice to use a butanol-production-pathway enzyme(s) that can use NADPH that is generated by the photo-driven electron transport process. *Clostridium saccharoperbutylacetonicum* butanol dehydrogenase (GenBank accession number: AB257439) and butyraldehyde dehydrogenase (GenBank: AY251646) are examples of a butanol-production-pathway enzyme that is capable of accepting either NADP(H) or NAD(H). Such a butanol-production-pathway enzyme that can use both NADPH and NADH (i.e., NAD(P)H) can also be selected for use in this 3-phosphoglycerate-branched and any of the other designer butanol-production pathway(s) (FIG. 1) as well. *Clostridium beijerinckii* Butyryl-CoA dehydrogenase (GenBank: AF494018) and 3-Hydroxybutyryl-CoA dehydrogenase (GenBank: AF494018) are examples of a

butanol-production-pathway enzyme that can accept only NAD(H). When a butanol-production-pathway enzyme that can only use NADH is employed, it may require an NADPH/NADH conversion mechanism in order for this 3-phosphoglycerate-branched butanol-production pathway to operate well. However, depending on the genetic backgrounds of a host organism, a conversion mechanism between NADPH and NADH may exist in the host so that NADPH and NADH may be interchangeably used in the organism. In addition, it is known that NADPH could be converted into NADH by a NADPH-phosphatase activity (Pattanayak and Chatterjee (1998) "Nicotinamide adenine dinucleotide phosphate phosphatase facilitates dark reduction of nitrate: regulation by nitrate and ammonia," *Biologia Plantarum* 41(1):75-84) and that NAD can be converted to NADP by a NAD kinase activity (Muto, Miyachi, Usuda, Edwards and Bassham (1981) "Light-induced conversion of nicotinamide adenine dinucleotide to nicotinamide adenine dinucleotide phosphate in higher plant leaves," *Plant Physiology* 68(2):324-328; Matsumura-Kadota, Muto, Miyachi (1982) "Light-induced conversion of NAD⁺ to NADP⁺ in *Chlorella* cells," *Biochimica Biophysica Acta* 679(2):300-300). Therefore, when enhanced NADPH/NADH conversion is desirable, the host may be genetically modified to enhance the NADPH phosphatase and NAD kinase activities. Thus, in one of the various embodiments, the photosynthetic butanol-producing designer plant, designer alga or plant cell further contains additional designer transgenes (FIG. 2B) to inducibly express one or more enzymes to facilitate the NADPH/NADH interconversion, such as the NADPH phosphatase and NAD kinase (GenBank: XM_001609395, XM_001324239), in the stroma of algal chloroplast.

[0117] Another embodiment that can provide an NADPH/NADH conversion mechanism is by properly selecting an appropriate branching point at the Calvin cycle for a designer butanol-production pathway to branch from. To confer this NADPH/NADH conversion mechanism by pathway design according to this embodiment, it is a preferred practice to branch a designer butanol-production pathway at or after the point of glyceraldehydes-3-phosphate of the Calvin cycle as shown in FIG. 1. In these pathway designs, the NADPH/NADH conversion is achieved essentially by a two-step mechanism: 1) Use of the step with the Calvin-cycle's glyceraldehyde-3-phosphate dehydrogenase, which uses NADPH in reducing 1,3-diphosphoglycerate to glyceraldehydes-3-phosphate; and 2) use of the step with the designer pathway's NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase 01, which produces NADH in oxidizing glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate. The net result of the two steps described above is the conversion of NADPH to NADH, which can supply the needed reducing power in the form of NADH for the designer butanol-production pathway(s). For step 1), use of the Calvin-cycle's NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase naturally in the host organism is usually sufficient. Consequently, introduction of a designer NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase 01 to work with the Calvin-cycle's NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase may confer the function of an NADPH/NADH conversion mechanism, which is needed for the 3-phosphoglycerate-branched butanol-production pathway (03-12 in FIG. 1) to operate well. For this reason, the designer NAD⁺-dependent glyceraldehyde-3-phosphate-dehydrogenase DNA construct (example 12, SEQ ID NO:12) is

used also as an NADPH/NADH-conversion designer gene (FIG. 2B) to support the 3-phosphoglycerate-branched butanol-production pathway (03-12 in FIG. 1) in one of the various embodiments. This also explains why it is important to use a NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase 01 to confer this two-step NADPH/NADH conversion mechanism for the designer butanol-production pathway (s). Therefore, in one of the various embodiments, it is also a preferred practice to use a NAD⁺-dependent glyceraldehyde-3-phosphate dehydrogenase, its isozymes, functional derivatives, analogs, designer modified enzymes and/or combinations thereof in the designer butanol-production pathway(s) as illustrated in FIG. 1.

iRNA Techniques to Further Tame Photosynthesis Regulation Mechanism

[0118] In another embodiment of the present invention, the host plant or cell is further modified to tame the Calvin cycle so that the host can directly produce liquid fuel butanol instead of synthesizing starch (glycogen in the case of oxyphotobacteria), celluloses and lignocelluloses that are often inefficient and hard for the biorefinery industry to use. According to the one of the various embodiments, inactivation of starch-synthesis activity is achieved by suppressing the expression of any of the key enzymes, such as, starch synthase (glycogen synthase in the case of oxyphotobacteria) 13, glucose-1-phosphate (G-1-P) adenylyltransferase 14, phosphoglucomutase 15, and hexose-phosphate-isomerase 16 of the starch-synthesis pathway which connects with the Calvin cycle (FIG. 1).

[0119] Introduction of a genetically transmittable factor that can inhibit the starch-synthesis activity that is in competition with designer butanol-production pathway(s) for the Calvin-cycle products can further enhance photosynthetic butanol production. In a specific embodiment, a genetically encoded-inhibitor (FIG. 2C) to the competitive starch-synthesis pathway is an interfering RNA (iRNA) molecule that specifically inhibits the synthesis of a starch-synthesis-pathway enzyme, for example, starch synthase 16, glucose-1-phosphate (G-1-P) adenylyltransferase 15, phosphoglucomutase 14, and/or hexose-phosphate-isomerase 13 as shown with numerical labels 13-16 in FIG. 1. The DNA sequences encoding starch synthase iRNA, glucose-1-phosphate (G-1-P) adenylyltransferase iRNA, a phosphoglucomutase iRNA and/or a G-P-isomerase iRNA, respectively, can be designed and synthesized based on RNA interference techniques known to those skilled in the art (Liszewski (Jun. 1, 2003) Progress in RNA interference, *Genetic Engineering News*, Vol. 23, number 11, pp. 1-59). Generally speaking, an interfering RNA (iRNA) molecule is anti-sense but complementary to a normal mRNA of a particular protein (gene) so that such iRNA molecule can specifically bind with the normal mRNA of the particular gene, thus inhibiting (blocking) the translation of the gene-specific mRNA to protein (Fire, Xu, Montgomery, Kostas, Driver, Mello (1998) "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*". *Nature* 391(6669):806-11; Dykxhoorn, Novina, Sharp (2003) "Killing the messenger: short RNAs that silence gene expression", *Nat Rev Mol Cell Biol.* 4(6):457-67).

[0120] Examples of a designer starch-synthesis iRNA DNA construct (FIG. 2C) are shown in SEQ ID NO: 27 and 28 listed. Briefly, SEQ ID NO: 27 presents example 27 for a designer Nial-promoter-controlled Starch-Synthase-iRNA DNA construct (860 bp) that includes a PCR FD primer

(sequence 1-20), a 262-bp Nia1 promoter (21-282), a Starch-Synthase iRNA sequence (283-617) consisting of start codon atg and a reverse complement sequence of two unique sequence fragments of a *Chlamydomonas reinhardtii* starch-synthase-mRNA sequence (GenBank: AF026422), a 223-bp RbcS2 terminator (618-850), and a PCR RE primer (851-860). Because of the use of a Nia1 promoter (21-282), this designer starch-synthesis iRNA gene is designed to be expressed only when needed to enhance photobiological butanol production in the presence of its specific inducer, nitrate (NO_3^-), which can be added into the culture medium as a fertilizer for induction of the designer organisms. The Starch-Synthase iRNA sequence (283-617) is designed to bind with the normal mRNA of the starch synthase gene, thus blocking its translation into a functional starch synthase. The inhibition of the starch/glycogen synthase activity at 16 in this manner is to channel more photosynthetic products of the Calvin cycle into the Calvin-cycle-branched butanol-production pathway(s) such as the glyceraldehydes-3-phosphate-branched butanol-production pathway 01-12 as illustrated in FIG. 1.

[0121] SEQ ID NO: 28 presents example 28 for a designer HydA1-promoter-controlled Starch-Synthase-iRNA DNA construct (1328 bp) that includes a PCR FD primer (sequence 1-20), a 282-bp HydA1 promoter (21-302), a designer Starch-Synthase iRNA sequence (303-1085), a 223-bp RbcS2 terminator (1086-1308), and a PCR RE primer (1309-1328). The designer Starch-Synthase-iRNA sequence (303-1085) comprises of: a 300-bp sense fragment (303-602) selected from the first 300-bp unique coding sequence of a *Chlamydomonas reinhardtii* starch synthase mRNA sequence (GenBank: AF026422), a 183-bp designer intron-like loop (603-785), and a 300-bp antisense sequence (786-1085) complement to the first 300-bp coding sequence of a *Chlamydomonas reinhardtii* starch-synthase-mRNA sequence (GenBank: AF026422). This designer Starch-Synthase-iRNA sequence (303-1085) is designed to inhibit the synthesis of starch synthase by the following two mechanisms. First, the 300-bp antisense complement iRNA sequence (corresponding to DNA sequence 786-1085) binds with the normal mRNA of the starch synthase gene, thus blocking its translation into a functional starch synthase. Second, the 300-bp antisense complement iRNA sequence (corresponding to DNA sequence 786-1085) can also bind with the 300-bp sense counterpart (corresponding to DNA sequence 303-602) in the same designer iRNA molecule, forming a hairpin-like double-stranded RNA structure with the 183-bp designer intron-like sequence (603-785) as a loop. Experimental studies have shown that this type of hairpin-like double-stranded RNA can also trigger post-transcriptional gene silencing (Fuhrmann, Stahlberg, Govorunova, Rank and Hegemann (2001) *Journal of Cell Science* 114:3857-3863). Because of the use of a HydA1 promoter (21-302), this designer starch-synthesis-iRNA gene is designed to be expressed only under anaerobic conditions when needed to enhance photobiological butanol production by channeling more photosynthetic products of the Calvin cycle into the butanol-production pathway (s) such as 01-12, 03-12, and/or 20-33 as illustrated in FIG. 1.

Designer Starch-Degradation and Glycolysis Genes

[0122] In yet another embodiment of the present invention, the photobiological butanol production is enhanced by incorporating an additional set of designer genes (FIG. 2D) that can facilitate starch/glycogen degradation and glycolysis in

combination with the designer butanol-production gene(s) (FIG. 2A). Such additional designer genes for starch degradation include, for example, genes coding for 17: amylase, starch phosphorylase, hexokinase, phosphoglucomutase, and for 18: glucose-phosphate-isomerase (G-P-isomerase) as illustrated in FIG. 1. The designer glycolysis genes encode chloroplast-targeted glycolysis enzymes: glucosephosphate isomerase 18, phosphofructose kinase 19, aldolase 20, triose phosphate isomerase 21, glyceraldehyde-3-phosphate dehydrogenase 22, phosphoglycerate kinase 23, phosphoglycerate mutase 24, enolase 25, and pyruvate kinase 26. The designer starch-degradation and glycolysis genes in combination with any of the butanol-production pathways shown in FIG. 1 can form additional pathway(s) from starch/glycogen to butanol (17-33). Consequently, co-expression of the designer starch-degradation and glycolysis genes with the butanol-production-pathway genes can enhance photobiological production of butanol as well. Therefore, this embodiment represents another approach to tame the Calvin cycle for enhanced photobiological production of butanol. In this case, some of the Calvin-cycle products flow through the starch synthesis pathway (13-16) followed by the starch/glycogen-to-butanol pathway (17-33) as shown in FIG. 1. In this case, starch/glycogen acts as a transient storage pool of the Calvin-cycle products before they can be converted to butanol. This mechanism can be quite useful in maximizing the butanol-production yield in certain cases. For example, at high sunlight intensity such as around noon, the rate of Calvin-cycle photosynthetic CO_2 fixation can be so high that may exceed the maximal rate capacity of a butanol-production pathway(s); use of the starch-synthesis mechanism allows temporary storage of the excess photosynthetic products to be used later for butanol production as well.

[0123] FIG. 1 also illustrates the use of a designer starch/glycogen-to-butanol pathway with designer enzymes (as labeled from 17 to 33) in combination with a Calvin-cycle-branched designer butanol-production pathway(s) such as the glyceraldehydes-3-phosphate-branched butanol-production pathway 01-12 for enhanced photobiological butanol production. Similar to the benefits of using the Calvin-cycle-branched designer butanol-production pathways, the use of the designer starch/glycogen-to-butanol pathway (17-33) can also help to convert the photosynthetic products to butanol before the sugars could be converted into other complicated biomolecules such as lignocellulosic biomasses which cannot be readily used by the biorefinery industries. Therefore, appropriate use of the Calvin-cycle-branched designer butanol-production pathway(s) (such as 01-12, 03-12, and/or 20-33) and/or the designer starch/glycogen-to-butanol pathway (17-33) may represent revolutionary inter alia technologies that can effectively bypass the bottleneck problems of the current biomass technology including the "lignocellulosic recalcitrance" problem.

[0124] Another feature is that a Calvin-cycle-branched designer butanol-production pathway activity (such as 01-12, 03-12, and/or 20-33) can occur predominantly during the days when there is light because it uses an intermediate product of the Calvin cycle which requires supplies of reducing power (NADPH) and energy (ATP) generated by the photosynthetic water splitting and the light-driven proton-translocation-coupled electron transport process through the thylakoid membrane system. The designer starch/glycogen-to-butanol pathway (17-33) which can use the surplus sugar that has been stored as starch/glycogen during photosynthesis can

operate not only during the days, but also at nights. Consequently, the use of a Calvin-cycle-branched designer butanol-production pathway (such as 01-12, 03-12, and/or 20-33) together with a designer starch/glycogen-to-butanol pathway (s) (17-33) as illustrated in FIG. 1 enables production of butanol both during the days and at nights.

[0125] Because the expression for both the designer starch/glycogen-to-butanol pathway(s) and the Calvin-cycle-branched designer butanol-production pathway(s) is controlled by the use of an inducible promoter such as an anaerobic hydrogenase promoter, this type of designer organisms is also able to grow photoautotrophically under aerobic (normal) conditions. When the designer photosynthetic organisms are grown and ready for photobiological butanol production, the cells are then placed under the specific inducing conditions such as under anaerobic conditions [or an ammonium-to-nitrate fertilizer use shift, if designer *Nia1/nirA* promoter-controlled butanol-production pathway(s) is used] for enhanced butanol production, as shown in FIGS. 1 and 3.

[0126] Examples of designer starch (glycogen)-degradation genes are shown in SEQ ID NO: 29-33 listed. Briefly, SEQ ID NO:29 presents example 29 for a designer Amylase DNA construct (1889 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp NR promoter (21-188), a 9-bp *Xho I NdeI* site (189-197), a 135-bp *RbcS2* transit peptide (198-332), an Amylase-encoding sequence (333-1616) selected and modified from a Barley alpha-amylase (GenBank: J04202A my46 expression tested in aleurone cells), a 21-bp Lumio-tag sequence (1617-1637), a 9-bp *XbaI* site (1638-1646), a 223-bp *RbcS2* terminator (1647-1869), and a PCR RE primer (1870-1889).

[0127] SEQ ID NO: 30 presents example 30 for a designer Starch-Phosphorylase DNA construct (3089 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp NR promoter (21-188), a 135-bp *RbcS2* transit peptide (189-323), a Starch Phosphorylase-encoding sequence (324-2846) selected and modified from a Citrus root starch-phosphorylase sequence (GenBank: AY098895, expression tested in citrus root), a 223-bp *RbcS2* terminator (2847-3069), and a PCR RE primer (3070-3089).

[0128] SEQ ID NO: 31 presents example 31 for a designer Hexose-Kinase DNA construct (1949 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp NR promoter (21-188), a 135-bp *RbcS2* transit peptide (189-323), a Hexose Kinase-encoding sequence (324-1706) selected and modified from *Ajellomyces capsulatus* hexokinase mRNA sequence (Genbank: XM_001541513), a 223-bp *RbcS2* terminator (1707-1929), and a PCR RE primer (1930-1949).

[0129] SEQ ID NO: 32 presents example 32 for a designer Phosphoglucomutase DNA construct (2249 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp NR promoter (21-188), a 135-bp *RbcS2* transit peptide (189-323), a Phosphoglucomutase-encoding sequence (324-2006) selected and modified from *Pichia stipitis* phosphoglucomutase sequence (GenBank: XM_001383281), a 223-bp *RbcS2* terminator (2007-2229), and a PCR RE primer (2230-2249).

[0130] SEQ ID NO: 33 presents example 33 for a designer Glucosephosphate-Isomerase DNA construct (2231 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp NR promoter (21-188), a 135-bp *RbcS2* transit peptide (189-323), a Glucosephosphate Isomerase-encoding sequence (324-1988) selected and modified from a *S. cerevisiae* phos-

phoglucoisomerase sequence (GenBank: M21696), a 223-bp *RbcS2* terminator (1989-2211), and a PCR RE primer (2212-2231).

[0131] The designer starch-degradation genes such as those shown in SEQ ID NO: 29-33 can be selected for use in combination with various designer butanol-production-pathway genes for construction of various designer starch-degradation butanol-production pathways such as the pathways shown in FIG. 1. For example, the designer genes shown in SEQ ID NOS: 1-12, 24-26, and 29-33 can be selected for construction of a *Nia1* promoter-controlled starch-to-butanol production pathway that comprises of the following designer enzymes: amylase, starch phosphorylase, hexokinase, phosphoglucomutase, glucosephosphate isomerase, phosphofructose kinase, fructose diphosphate aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase, pyruvate-NADP⁺ oxidoreductase (or pyruvate-ferredoxin oxidoreductase), thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase. This starch/glycogen-to-butanol pathway 17-33 may be used alone and/or in combinations with other butanol-production pathway(s) such as the 3-phosphoglycerate-branched butanol-production pathway 03-12 as illustrated in FIG. 1.

Distribution of Designer Butanol-Production Pathways Between Chloroplast and Cytoplasm

[0132] In yet another embodiment of the present invention, photobiological butanol productivity is enhanced by a selected distribution of the designer butanol-production pathway(s) between chloroplast and cytoplasm in a eukaryotic plant cell. That is, not all the designer butanol-production pathway(s) (FIG. 1) have to operate in the chloroplast; when needed, part of the designer butanol-production pathway(s) can operate in cytoplasm as well. For example, in one of the various embodiments, a significant part of the designer starch-to-butanol pathway activity from dihydroxyacetone phosphate to butanol (21-33) is designed to occur at the cytoplasm while the steps from starch to dihydroxyacetone phosphate (17-20) are in the chloroplast. In this example, the linkage between the chloroplast and cytoplasm parts of the designer pathway is accomplished by use of the triose phosphate-phosphate translocator, which facilitates translocation of dihydroxyacetone across the chloroplast membrane. By use of the triose phosphate-phosphate translocator, it also enables the glyceraldehyde-3-phosphate-branched designer butanol-production pathway to operate not only in chloroplast, but also in cytoplasm as well. The cytoplasm part of the designer butanol-production pathway can be constructed by use of designer butanol-production pathway genes (DNA constructs of FIG. 2A) with their chloroplast-targeting sequence omitted as shown in FIG. 2E.

Designer Oxyphotobacteria with Designer Butanol-Production Pathways in Cytoplasm

[0133] In prokaryotic photosynthetic organisms such as blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria), which typically contain photosynthetic thylakoid membrane but no chloroplast structure, the Calvin cycle is located in the cytoplasm. In this special case, the entire designer butanol-production pathway(s) (FIG. 1) including (but not limited to) the glyceraldehyde-3-phosphate branched butanol-production pathway (01-12), the

3-phosphoglycerate-branched butanol-production pathway (03-12), the fructose-1,6-diphosphate-branched pathway (20-33), the fructose-6-phosphate-branched pathway (19-33), and the starch (or glycogen)-to-butanol pathways (17-33) are adjusted in design to operate with the Calvin cycle in the cytoplasm of a blue-green alga. The construction of the cytoplasm designer butanol-production pathways can be accomplished by use of designer butanol-production pathway genes (DNA construct of FIG. 2A) with their chloroplast-targeting sequence all omitted. When the chloroplast-targeting sequence is omitted in the designer DNA construct(s) as illustrated in FIG. 2E, the designer gene(s) is transcribed and translated into designer enzymes in the cytoplasm whereby conferring the designer butanol-production pathway(s). The designer gene(s) can be incorporated into the chromosomal and/or plasmid DNA in host blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria) by using the techniques of gene transformation known to those skilled in the art. It is a preferred practice to integrate the designer genes through an integrative transformation into the chromosomal DNA that can usually provide better genetic stability for the designer genes. In oxyphotobacteria such as cyanobacteria, integrative transformation can be achieved through a process of homologous DNA double recombination into the host's chromosomal DNA using a designer DNA construct as illustrated in FIG. 2F, which typically, from the 5' upstream to the 3' downstream, consists of: recombination site 1, a designer butanol-production-pathway gene(s), and recombination site 2. This type of DNA constructs (FIG. 2F) can be delivered into oxyphotobacteria (blue-green algae) with a number of available genetic transformation techniques including electroporation, natural transformation, and/or conjugation. The transgenic designer organisms created from blue-green algae are also called designer blue-green algae (designer oxyphotobacteria including designer cyanobacteria and designer oxychlorobacteria).

[0134] Examples of designer oxyphotobacterial butanol-production-pathway genes are shown in SEQ ID NO: 34-45 listed. Briefly, SEQ ID NO:34 presents example 34 for a designer oxyphotobacterial Butanol Dehydrogenase DNA construct (1709 bp) that includes a PCR FD primer (sequence 1-20), a 400-bp nitrite reductase (*nirA*) promoter from *Thermosynechococcus elongatus* BP-1 (21-420), an enzyme-encoding sequence (421-1569) selected and modified from a *Clostridium saccharoperbutylacetonicum* Butanol Dehydrogenase sequence (AB257439), a 120-bp *rbcS* terminator from *Thermosynechococcus elongatus* BP-1 (1570-1689), and a PCR RE primer (1690-1709) at the 3' end.

[0135] SEQ ID NO:35 presents example 35 for a designer oxyphotobacterial Butyraldehyde Dehydrogenase DNA construct (1967 bp) that includes a PCR FD primer (sequence 1-20), a 400-bp *Thermosynechococcus elongatus* BP-1 nitrite reductase *nirA* promoter (21-420), an enzyme-encoding sequence (421-1827) selected and modified from a *Clostridium saccharoperbutylacetonicum* Butyraldehyde Dehydrogenase sequence (AY251646), a 120-bp *rbcS* terminator from *Thermosynechococcus* (1828-1947), and a PCR RE primer (1948-1967).

[0136] SEQ ID NO:36 presents example 36 for a designer oxyphotobacterial Butyryl-CoA Dehydrogenase DNA construct (1602 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *Thermosynechococcus elongatus* BP-1 nitrate reductase promoter (21-325), a Butyryl-CoA Dehydrogenase encoding sequence (326-1422) selected/modified

from the sequences of a *Clostridium beijerinckii* Butyryl-CoA Dehydrogenase (AF494018), a 120-bp *Thermosynechococcus rbcS* terminator (1423-1582), and a PCR RE primer (1583-1602).

[0137] SEQ ID NO:37 presents example 37 for a designer oxyphotobacterial Crotonase DNA construct (1248 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *Thermosynechococcus elongatus* BP-1 nitrate reductase promoter (21-325), a Crotonase-encoding sequence (326-1108) selected/modified from the sequences of a *Clostridium beijerinckii* Crotonase (GenBank: AF494018), 120-bp *Thermosynechococcus elongatus* BP-1 *rbcS* terminator (1109-1228), and a PCR RE primer (1229-1248).

[0138] SEQ ID NO:38 presents example 38 for a designer oxyphotobacterial 3-Hydroxybutyryl-CoA Dehydrogenase DNA construct (1311 bp) that include of a PCR FD primer (sequence 1-20), a 305-bp *nirA* promoter (21-325), a 3-Hydroxybutyryl-CoA Dehydrogenase-encoding sequence (326-1171) selected/modified from a *Clostridium beijerinckii* 3-Hydroxybutyryl-CoA Dehydrogenase sequence Crotonase (GenBank: AF494018), a 120-bp *Thermosynechococcus rbcS* terminator (1172-1291), and a PCR RE primer (1292-1311).

[0139] SEQ ID NO:39 presents example 39 for a designer oxyphotobacterial Thiolase DNA construct (1665 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *Thermosynechococcus nirA* promoter (21-325), a Thiolase-encoding sequence (326-1525) selected from a *Butyrivibrio fibrisolvens* Thiolase sequence (AB190764), a 120-bp *Thermosynechococcus rbcS* terminator (1526-1645), and a PCR RE primer (1646-1665).

[0140] SEQ ID NO:40 presents example 40 for a designer oxyphotobacterial Pyruvate-Ferredoxin Oxidoreductase DNA construct (4071 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *nirA* promoter from *Thermosynechococcus elongatus* BP-1 (21-325), a Pyruvate-Ferredoxin Oxidoreductase-encoding sequence (326-3931) selected/modified from the sequences of a *Mastigamoeba balamuthi* Pyruvate-ferredoxin oxidoreductase (GenBank: AY101767), a 120-bp *rbcS* terminator from *Thermosynechococcus elongatus* BP-1 (3932-4051), and a PCR RE primer (4052-4071).

[0141] SEQ ID NO:41 presents example 41 for a designer oxyphotobacterial Pyruvate Kinase DNA construct (1806 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *nirA* promoter from *Thermosynechococcus* (21-325), a pyruvate kinase-encoding sequence (326-1666) selected/modified from a *Thermoproteus tenax* pyruvate kinase (GenBank: AF065890), a 120-bp *Thermosynechococcus rbcS* terminator (1667-1786), and a PCR RE primer (1787-1806).

[0142] SEQ ID NO:42 presents example 42 for a designer oxyphotobacterial Enolase DNA construct (1696 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus* (21-251), an enolase-encoding sequence (252-1556) selected/modified from the sequences of a *Chlamydomonas* cytosolic enolase (GenBank: X66412, P31683), a 120-bp *rbcS* terminator from *Thermosynechococcus* (1557-1676), and a PCR RE primer (1677-1696).

[0143] SEQ ID NO:43 presents example 43 for a designer oxyphotobacterial Phosphoglycerate-Mutase DNA construct (2029 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP-1 (21-251), a phosphoglycerate-mutase encoding sequence (252-1889) selected/modified from the sequences

of a *Pelotomaculum thermopropionicum* SI phosphoglycerate mutase (GenBank: YP_001213270), a 120-bp *Thermosynechococcus* rbcS terminator (1890-2009), and a PCR RE primer (2010-2029).

[0144] SEQ ID NO:44 presents example 44 for a designer oxyphotobacterial Phosphoglycerate Kinase DNA construct (1687 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP-1 (21-251), a phosphoglycerate-kinase-encoding sequence (252-1433) selected from *Pelotomaculum thermopropionicum* SI phosphoglycerate kinase (BAF60903), a 234-bp *Thermosynechococcus elongatus* BP-1 rbcS terminator (1434-1667), and a PCR RE primer (1668-1687).

[0145] SEQ ID NO:45 presents example 45 for a designer oxyphotobacterial Glyceraldehyde-3-Phosphate Dehydrogenase DNA construct (1514 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *Thermosynechococcus elongatus* BP-1 nirA promoter (21-325), an enzyme-encoding sequence (326-1260) selected and modified from *Blastochloris viridis* NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase (CAC80993), a 234-bp rbcS terminator from *Thermosynechococcus elongatus* BP-1 (1261-1494), and a PCR RE primer (1495-1514).

[0146] The designer oxyphotobacterial genes such as those shown in SEQ ID NO: 34-45 can be selected for use in full or in part, and/or in combination with various other designer butanol-production-pathway genes for construction of various designer oxyphotobacterial butanol-production pathways such as the pathways shown in FIG. 1. For example, the designer genes shown in SEQ ID NOS: 34-45 can be selected for construction of an oxyphotobacterial nirA promoter-controlled and glyceraldehyde-3-phosphate-branched butanol-production pathway (01-12) that comprises of the following designer enzymes: NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 01, phosphoglycerate kinase 02, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, pyruvate-ferredoxin oxidoreductase (or pyruvate-NADP⁺ oxidoreductase) 06, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, and butanol dehydrogenase 12. Use of these designer oxyphotobacterial butanol-production-pathway genes (SEQ ID NOS: 34-45) in a thermophilic and/or thermotolerant cyanobacterium may represent a thermophilic and/or thermotolerant butanol-producing oxyphotobacterium. For example, use of these designer genes (SEQ ID NOS: 34-45) in a thermophilic/thermotolerant cyanobacterium such as *Thermosynechococcus elongatus* BP-1 may represent a designer thermophilic/thermotolerant butanol-producing cyanobacterium such as a designer butanol-producing *Thermosynechococcus*.

Further Host Modifications to Help Ensure Biosafety

[0147] The present invention also provides biosafety-guarded photosynthetic biofuel (e.g., butanol and/or related higher alcohols) production methods based on cell-division-controllable designer transgenic plants (such as algae and oxyphotobacteria) or plant cells. For example, the cell-division-controllable designer photosynthetic organisms (FIG. 3) are created through use of a designer biosafety-control gene (s) (FIG. 2G) in conjunction with the designer butanol-production-pathway gene(s) (FIGS. 2A-2F) such that their cell division and mating function can be controllably stopped to provide better biosafety features.

[0148] In one of the various embodiments, a fundamental feature is that a designer cell-division-controllable photosynthetic organism (such as an alga, plant cell, or oxyphotobacterium) contains two key functions (FIG. 3A): a designer biosafety mechanism(s) and a designer biofuel-production pathway(s). As shown in FIG. 3B, the designer biosafety feature(s) is conferred by a number of mechanisms including: (1) the inducible insertion of designer proton-channels into cytoplasm membrane to permanently disable any cell division and mating capability, (2) the selective application of designer cell-division-cycle regulatory protein or interference RNA (iRNA) to permanently inhibit the cell division cycle and preferably keep the cell at the G₁ phase or G₀ state, and (3) the innovative use of a high-CO₂-requiring host photosynthetic organism for expression of the designer biofuel-production pathway(s). Examples of the designer biofuel-production pathway(s) include the designer butanol-production pathway(s), which work with the Calvin cycle to synthesize biofuel such as butanol directly from carbon dioxide (CO₂) and water (H₂O). The designer cell-division-control technology can help ensure biosafety in using the designer organisms for photosynthetic biofuel production. Accordingly, this embodiment provides, inter alia, biosafety-guarded methods for producing biofuel (e.g., butanol and/or related higher alcohols) based on a cell-division-controllable designer biofuel-producing alga, cyanobacterium, oxychlorobacterium, plant or plant cells.

[0149] In one of the various embodiments, a cell-division-controllable designer butanol-producing eukaryotic alga or plant cell is created by introducing a designer proton-channel gene (FIG. 2H) into a host alga or plant cell (FIG. 3B). SEQ ID NO: 46 presents example 46 for a detailed DNA construct of a designer Nia1-promoter-controlled proton-channel gene (609 bp) that includes a PCR FD primer (sequence 1-20), a 262-bp nitrate reductase Nia1 promoter (21-282), a Melittin proton-channel encoding sequence (283-366), a 223-bp RbcS2 terminator (367-589), and a PCR RE primer (590-609).

[0150] The expression of the designer proton-channel gene (FIG. 2H) is controlled by an inducible promoter such as the nitrate reductase (Nia1) promoter, which can also be used to control the expression of a designer biofuel-production-pathway gene(s). Therefore, before the expression of the designer gene(s) is induced, the designer organism can grow photoautotrophically using CO₂ as the carbon source and H₂O as the source of electrons just like wild-type organism. When the designer organism culture is grown and ready for photobiological production of biofuels, the cell culture is then placed under a specific inducing condition (such as by adding nitrate into the culture medium if the nitrate reductase (Nia1) promoter is used as an inducible promoter) to induce the expression of both the designer proton-channel gene and the designer biofuel-production-pathway gene(s). The expression of the proton-channel gene is designed to occur through its transcription in the nucleus and its translation in the cytosol. Because of the specific molecular design, the expressed proton channels are automatically inserted into the cytoplasm membrane, but leave the photosynthetic thylakoid membrane intact. The insertion of the designer proton channels into cytoplasm membrane collapses the proton gradient across the cytoplasm membrane so that the cell division and mating function are permanently disabled. However, the photosynthetic thylakoid membrane inside the chloroplast is kept intact (functional) so that the designer biofuel-production-

pathway enzymes expressed into the stroma region can work with the Calvin cycle for photobiological production of biofuels from CO₂ and H₂O. That is, when both the designer proton-channel gene and the designer biofuel-production-pathway gene(s) are turned on, the designer organism becomes a non-reproducible cell for dedicated photosynthetic production of biofuels. Because the cell division and mating function are permanently disabled (killed) at this stage, the designer-organism culture is no longer a living matter except its catalytic function for photochemical conversion of CO₂ and H₂O into a biofuel. It will no longer be able to mate or exchange any genetic materials with any other cells, even if it somehow comes in contact with a wild-type cell as it would be the case of an accidental release into the environments.

[0151] According to one of the various embodiments, the nitrate reductase (Nia1) promoter or nitrite reductase (nirA) promoter is a preferred inducible promoter for use to control the expression of the designer genes. In the presence of ammonium (but not nitrate) in culture medium, for example, a designer organism with Nia1-promoter-controlled designer proton-channel gene and biofuel-production-pathway gene(s) can grow photoautotrophically using CO₂ as the carbon source and H₂O as the source of electrons just like a wild-type organism. When the designer organism culture is grown and ready for photobiological production of biofuels, the expression of both the designer proton-channel gene and the designer biofuel-production-pathway gene(s) can then be induced by adding some nitrate fertilizer into the culture medium. Nitrate is widely present in soils and nearly all surface water on Earth. Therefore, even if a Nia1-promoter-controlled designer organism is accidentally released into the natural environment, it will soon die since the nitrate in the environment will trigger the expression of a Nia1-promoter-controlled designer proton-channel gene which inserts proton-channels into the cytoplasm membrane thereby killing the cell. That is, a designer photosynthetic organism with Nia1-promoter-controlled proton-channel gene is programmed to die as soon as it sees nitrate in the environment. This characteristic of cell-division-controllable designer organisms with Nia1-promoter-controlled proton-channel gene provides an added biosafety feature.

[0152] The art in constructing proton-channel gene (FIG. 2H) with a thylakoid-membrane targeting sequence has recently been disclosed [James W. Lee (2007). Designer proton-channel transgenic algae for photobiological hydrogen production, PCT International Publication Number: WO 2007/134340 A2]. In the present invention of creating a cell-division-controllable designer organism, the thylakoid-membrane-targeting sequence must be omitted in the proton-channel gene design. For example, the essential components of a Nia1-promoter-controlled designer proton-channel gene can simply be a Nia1 promoter linked with a proton-channel-encoding sequence (without any thylakoid-membrane-targeting sequence) so that the proton channel will insert into the cytoplasm membrane but not into the photosynthetic thylakoid membrane.

[0153] According to one of the various embodiments, it is a preferred practice to use the same inducible promoter such as the Nia1 promoter to control the expression of both the designer proton-channel gene and the designer biofuel-production pathway genes. In this way, the designer biofuel-production pathway(s) can be inducibly expressed simulta-

neously with the expression of the designer proton-channel gene that terminates certain cellular functions including cell division and mating.

[0154] In one of the various embodiments, an inducible promoter that can be used in this designer biosafety embodiment is selected from the group consisting of the hydrogenase promoters [HydA1 (Hyd1) and HydA2, accession number: AJ308413, AF289201, AY090770], the Cyc6 gene promoter, the Cpx1 gene promoter, the heat-shock protein promoter HSP70A, the CabII-1 gene (accession number M24072) promoter, the Ca1 gene (accession number P20507) promoter, the Ca2 gene (accession number P24258) promoter, the nitrate reductase (Nia1) promoter, the nitrite-reductase-gene (nirA) promoters, the bidirectional-hydrogenase-gene hox promoters, the light- and heat-responsive groE promoters, the Rubisco-operon rbcL promoters, the metal (zinc)-inducible smt promoter, the iron-responsive idiA promoter, the redox-responsive crhR promoter, the heat-shock-gene hsp16.6 promoter, the small heat-shock protein (Hsp) promoter, the CO₂-responsive carbonic-anhydrase-gene promoters, the green/red light responsive cpcB2A2 promoter, the UV-light responsive lexA, recA and ruvB promoters, the nitrate-reductase-gene (narB) promoters, and combinations thereof.

[0155] In another embodiment, a cell-division-controllable designer photosynthetic organism is created by use of a carbonic anhydrase deficient mutant or a high-CO₂-requiring mutant as a host organism to create the designer biofuel-production organism. High-CO₂-requiring mutants that can be selected for use in this invention include (but not limited to): *Chlamydomonas reinhardtii* carbonic-anhydrase-deficient mutant 12-1C(CC-1219 ca1 mt-), *Chlamydomonas reinhardtii* cia3 mutant (*Plant Physiology* 2003, 132:2267-2275), the high-CO₂-requiring mutant M3 of *Synechococcus* sp. Strain PCC 7942, or the carboxysome-deficient cells of *Synechocystis* sp. PCC 6803 (*Plant Biol* (Stuttg) 2005, 7:342-347) that lacks the CO₂-concentrating mechanism can grow photoautotrophically only under elevated CO₂ concentration level such as 0.2-3% CO₂.

[0156] Under atmospheric CO₂ concentration level (380 ppm), the carbonic anhydrase deficient or high-CO₂-requiring mutants commonly cannot survive. Therefore, the key concept here is that a high-CO₂-requiring designer biofuel-production organism that lacks the CO₂ concentrating mechanism will be grown and used for photobiological production of biofuels always under an elevated CO₂ concentration level (0.2-5% CO₂) in a sealed bioreactor with CO₂ feeding. Such a designer transgenic organism cannot survive when it is exposed to an atmospheric CO₂ concentration level (380 ppm=0.038% CO₂) because its CO₂-concentrating mechanism (CCM) for effective photosynthetic CO₂ fixation has been impaired by the mutation. Even if such a designer organism is accidentally released into the natural environment, its cell will soon not be able to divide or mate, but die quickly of carbon starvation since it cannot effectively perform photosynthetic CO₂ fixation at the atmospheric CO₂ concentration (380 ppm). Therefore, use of such a high-CO₂-requiring mutant as a host organism for the genetic transformation of the designer biofuel-production-pathway gene(s) represents another way in creating the envisioned cell-division-controllable designer organisms for biosafety-guarded photobiological production of biofuels from CO₂ and H₂O. No designer proton-channel gene is required here.

[0157] In another embodiment, a cell-division-controllable designer organism (FIG. 3B) is created by use of a designer

cell-division-cycle regulatory gene as a biosafety-control gene (FIG. 2G) that can control the expression of the cell-division-cycle (*cdc*) genes in the host organism so that it can inducibly turn off its reproductive functions such as permanently shutting off the cell division and mating capability upon specific induction of the designer gene.

[0158] Biologically, it is the expression of the natural *cdc* genes that controls the cell growth and cell division cycle in cyanobacteria, algae, and higher plant cells. The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes during the S phase (S for synthesis) and then segregate the copies precisely into two genetically identical daughter cells during the M phase (M for mitosis). Mitosis begins typically with chromosome condensation: the duplicated DNA strands, packaged into elongated chromosomes, condense into the much-more compact chromosomes required for their segregation. The nuclear envelope then breaks down, and the replicated chromosomes, each consisting of a pair of sister chromatids, become attached to the microtubules of the mitotic spindle. As mitosis proceeds, the cell pauses briefly in a state called metaphase, when the chromosomes are aligned at the equator of the mitotic spindle, poised for segregation. The sudden segregation of sister chromatids marks the beginning of anaphase during which the chromosomes move to opposite poles of the spindle, where they decondense and reform intact nuclei. The cell is then pinched into two by cytoplasmic division (cytokinesis) and the cell division is then complete. Note, most cells require much more time to grow and double their mass of proteins and organelles than they require to replicate their DNA (the S phase) and divide (the M phase). Therefore, there are two gap phases: a G_1 phase between M phase and S phase, and a G_2 phase between S phase and mitosis. As a result, the eukaryotic cell cycle is traditionally divided into four sequential phases: G_1 , S, G_2 , and M. Physiologically, the two gap phases also provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable and preparation are complete before the cell commits itself to the major upheavals of S phase and mitosis. The G_1 phase is especially important in this aspect. Its length can vary greatly depending on external conditions and extracellular signals from other cells. If extracellular conditions are unfavorable, for example, cells delay progress through G_1 and may even enter a specialized resting state known as G_0 (G_0 zero), in which they remain for days, weeks, or even for years before resuming proliferation. Indeed, many cells remain permanently in G_0 state until they die.

[0159] In one of the various embodiments, a designer gene (s) that encodes a designer *cdc*-regulatory protein or a specific *cdc*-iRNA is used to inducibly inhibit the expression of certain *cdc* gene(s) to stop cell division and disable the mating capability when the designer gene(s) is triggered by a specific inducing condition. When the cell-division-controllable designer culture is grown and ready for photosynthetic production of biofuels, for example, it is a preferred practice to induce the expression of a specific designer *cdc*-iRNA gene (s) along with induction of the designer biofuel-production-pathway gene(s) so that the cells will permanently halt at the G_1 phase or G_0 state. In this way, the grown designer-organism cells become perfect catalysts for photosynthetic production of biofuels from CO_2 and H_2O while their functions of cell division and mating are permanently shut off at the G_1 phase or G_0 state to help ensure biosafety.

[0160] Use of the biosafety embodiments with various designer biofuel-production-pathways genes listed in SEQ ID NOS: 1-45 (and 58-165) can create various biosafety-guarded photobiological biofuel producers (FIGS. 3A, 3B, and 3C). Note, SEQ ID NOS: 46 and 1-12 (examples 1-12) represent an example for a cell-division-controllable designer eukaryotic organism such as a cell-division-controllable designer alga (e.g., *Chlamydomonas*) that contains a designer *Nia1*-promoter-controlled proton-channel gene (SEQ ID NO: 46) and a set of designer *Nia1*-promoter-controlled butanol-production-pathway genes (SEQ ID NOS: 1-12). Because the designer proton-channel gene and the designer biofuel-production-pathway gene(s) are all controlled by the same *Nia1*-promoter sequences, they can be simultaneously expressed upon induction by adding nitrate fertilizer into the culture medium to provide the biosafety-guarded photosynthetic biofuel-producing capability as illustrated in FIG. 3B. Use of the designer *Nia1*-promoter-controlled butanol-production-pathway genes (SEQ ID NOS: 1-12) in a high CO_2 -requiring host photosynthetic organism, such as *Chlamydomonas reinhardtii* carbonic-anhydrase-deficient mutant 12-1C (CC-1219 ca1 mt-) or *Chlamydomonas reinhardtii* *cia3* mutant, represents another example in creating a designer cell-division-controllable photosynthetic organism to help ensure biosafety.

[0161] This designer biosafety feature may be useful to the production of other biofuels such as biooils, biohydrogen, ethanol, and intermediate products as well. For example, this biosafety embodiment in combination with a set of designer ethanol-production-pathway genes such as those shown SEQ ID NOS: 47-53 can represent a cell-division-controllable ethanol producer (FIG. 3C). Briefly, SEQ ID NO: 47 presents example 47 for a detailed DNA construct (1360 base pairs (bp)) of a *nirA*-promoter-controlled designer NAD-dependent Glyceraldehyde-3-Phosphate-Dehydrogenase gene including: a PCR FD primer (sequence 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* sp. (freshwater cyanobacterium) nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1032) selected and modified from a *Cyanidium caldarium* cytosolic NAD-dependent glyceraldehyde-3-phosphate-dehydrogenase sequence (GenBank accession number: CAC85917), a 308-bp *Synechococcus rbcS* terminator (1033-1340), and a PCR RE primer (1341-1360) at the 3' end.

[0162] SEQ ID NO: 48 presents example 48 for a designer *nirA*-promoter-controlled Phosphoglycerate Kinase DNA construct (1621 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* sp. strain PCC 7942 nitrite-reductase *nirA* promoter (21-108), a phosphoglycerate-kinase-encoding sequence (109-1293) selected from a *Geobacillus kaustophilus* phosphoglycerate-kinase sequence (GenBank: BAD77342), a 308-bp *Synechococcus rbcS* terminator (1294-1601), and a PCR RE primer (1602-1621).

[0163] SEQ ID NO: 49 presents example 49 for a designer *nirA*-promoter-controlled Phosphoglycerate-Mutase DNA construct (1990 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* sp. strain PCC 7942 nitrite-reductase *nirA* promoter (21-108), a 9-bp *Xho I NdeI* site (109-117), a phosphoglycerate-mutase encoding sequence (118-1653) selected from the sequences of a *Caldicellulosiruptor saccharolyticus* DSM 8903 phosphoglycerate mutase (GenBank: ABP67536), a 9-bp *XbaI* site (1654-1662), a 308-bp *Synechococcus* sp. strain PCC 7942 *rbcS* terminator (1663-1970), and a PCR RE primer (1971-1990).

[0164] SEQ ID NO: 50 presents example 50 for a designer nirA-promoter-controlled Enolase DNA construct (1765 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* sp. strain PCC 7942 nitrite reductase nirA promoter (21-108), a 9-bp Xho I NdeI site (109-117), an enolase-encoding sequence (118-1407) selected from the sequence of a *Cyanothece* sp. CCY0110 enolase (GenBank: ZP_01727912), a 21-bp Lumio-tag-encoding sequence (1408-1428), a 9-bp XbaI site (1429-1437) containing a stop codon, a 308-bp *Synechococcus* rbcS terminator (1438-1745), and a PCR RE primer (1746-1765) at the 3' end.

[0165] SEQ ID NO: 51 presents example 51 for a designer nirA-promoter-controlled Pyruvate Kinase DNA construct (1888 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* nitrite reductase nirA promoter (21-108), a 9-bp Xho I NdeI site (109-117), a Pyruvate-Kinase-encoding sequence (118-1530) selected from a *Selenomonas ruminantium* Pyruvate Kinase sequence (GenBank: AB037182), a 21-bp Lumio-tag sequence (1531-1551), a 9-bp XbaI site (1552-1560), a 308-bp *Synechococcus* rbcS terminator (1561-1868), and a PCR RE primer (1869-1888).

[0166] SEQ ID NO: 52 presents example 52 for a designer nirA-promoter-controlled Pyruvate Decarboxylase DNA construct (2188 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* nitrite reductase nirA promoter (21-108), a 9-bp Xho I NdeI site (109-117), a Pyruvate-Decarboxylase-encoding sequence (118-1830) selected from the sequences of a *Pichia stipitis* pyruvate-decarboxylase sequence (GenBank: XM_001387668), a 21-bp Lumio-tag sequence (1831-1851), a 9-bp XbaI site (1852-1860), a 308-bp *Synechococcus* rbcS terminator (1861-2168), and a PCR RE primer (2169-2188) at the 3' end.

[0167] SEQ ID NO: 53 presents example 53 for a nirA-promoter-controlled designer NAD(P)H-dependent Alcohol Dehydrogenase DNA construct (1510 bp) that includes a PCR FD primer (sequence 1-20), a 88-bp *Synechococcus* nitrite-reductase nirA promoter (21-108), a NAD(P)H dependent Alcohol-Dehydrogenase-encoding sequence (109-1161) selected/modified (its mitochondrial signal peptide sequence removed) from the sequence of a *Kluyveromyces lactis* alcohol dehydrogenase (ADH3) gene (GenBank: X62766), a 21-bp Lumio-tag sequence (1162-1182), a 308-bp *Synechococcus* rbcS terminator (1183-1490), and a PCR RE primer (1491-1510) at the 3' end.

[0168] Note, SEQ ID NOS: 47-53 (DNA-construct examples 47-53) represent a set of designer nirA-promoter-controlled ethanol-production-pathway genes that can be used in oxyphotobacteria such as *Synechococcus* sp. strain PCC 7942. Use of this set of designer ethanol-production-pathway genes in a high-CO₂-requiring cyanobacterium such as the *Synechococcus* sp. Strain PCC 7942 mutant M3 represents another example of cell-division-controllable designer cyanobacterium for biosafety-guarded photosynthetic production of biofuels from CO₂ and H₂O.

More on Designer Calvin-Cycle-Channeled Production of Butanol and Related Higher Alcohols

[0169] The present invention further discloses designer Calvin-cycle-channeled and photosynthetic-NADPH (reduced nicotinamide adenine dinucleotide phosphate)-enhanced pathways, associated designer DNA constructs (designer genes) and designer transgenic photosynthetic organisms for photobiological production of butanol and related higher alcohols from carbon dioxide and water. In this

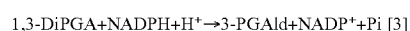
context throughout this specification as mentioned before, a "higher alcohol" or "related higher alcohol" refers to an alcohol that comprises at least four carbon atoms, including both straight and branched higher alcohols such as 1-butanol and 2-methyl-1-butanol. The Calvin-cycle-channeled and photosynthetic-NADPH-enhanced pathways are constructed with designer enzymes expressed through use of designer genes in host photosynthetic organisms such as algae and oxyphotobacteria (including cyanobacteria and oxychlorobacteria) organisms for photobiological production of butanol and related higher alcohols. The said butanol and related higher alcohols are selected from the group consisting of: 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, and 6-methyl-1-heptanol. The designer photosynthetic organisms such as designer transgenic algae and oxyphotobacteria (including cyanobacteria and oxychlorobacteria) comprise designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathway gene(s) and biosafety-guarding technology for enhanced photobiological production of butanol and related higher alcohols from carbon dioxide and water.

[0170] Photosynthetic water splitting and its associated proton gradient-coupled electron transport process generates chemical energy intermediate in the form of adenosine triphosphate (ATP) and reducing power in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH). However, certain butanol-related metabolic pathway enzymes such as the NADH-dependent butanol dehydrogenase (GenBank accession numbers: YP_148778, NP_561774, AAG23613, ZP_05082669, ADO12118, ADC48983) can use only reduced nicotinamide adenine dinucleotide (NADH) but not NADPH. Therefore, to achieve a true coupling of a designer pathway with the Calvin cycle for photosynthetic production of butanol and related higher alcohols, it is a preferred practice to use an effective NADPH/NADH conversion mechanism and/or NADPH-using enzyme(s) (such as NADPH-dependent enzymes) in construction of a compatible designer pathway(s) to couple with the photosynthesis/Calvin-cycle process in accordance with the present invention.

[0171] According to one of the various embodiments, a number of various designer Calvin-cycle-channeled pathways can be created by use of an NADPH/NADH conversion mechanism in combination with certain amino-acids-metabolic pathways for production of butanol and higher alcohols from carbon dioxide and water. The Calvin-cycle-channeled and photosynthetic-NADPH-enhanced pathways are constructed typically with designer enzymes that are selectively expressed through use of designer genes in a host photosynthetic organism such as a host alga or oxyphotobacterium for production of butanol and higher alcohols. A list of exemplary enzymes that can be selected for use in construction of the Calvin-cycle-channeled and photosynthetic-NADPH-enhanced pathways are presented in Table 1. As shown in FIGS. 4-10, the net results of the designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathways in working with the Calvin cycle are production of butanol and related higher alcohols from carbon dioxide (CO₂) and water (H₂O) using photosynthetically generated ATP (Adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate). A significant feature is the innovative utilization of an NADPH-dependent glyceraldehyde-3-phosphate

dehydrogenase 34 and a nicotinamide adenine dinucleotide (NAD)-dependent glyceraldehyde-3-phosphate dehydrogenase 35 to serve as a NADPH/NADH conversion mechanism that can convert certain amount of photosynthetically generated NADPH to NADH which can then be used by NADH-requiring pathway enzymes such as an NADH-dependent alcohol dehydrogenase 43 (examples of its encoding gene with GenBank accession numbers are: BAB59540, CAA89136, NP_148480) for production of butanol and higher alcohols.

[0172] More specifically, an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 (e.g., GenBank accession numbers: ADC37857, ADC87332, YP_003471459, ZP_04395517, YP_003287699, ZP_07004478, ZP_04399616) catalyzes the following reaction that uses NADPH in reducing 1,3-Diphosphoglycerate (1,3-DiPGA) to 3-Phosphoglyaldehyde (3-PGAld) and inorganic phosphate (Pi):



Meanwhile, an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 (e.g., GenBank: ADM41489,

YP_003095198, ADC36961, ZP_07003925, ACQ61431, YP_002285269, ADN80469, ACI60574) catalyzes the oxidation of 3-PGAld by oxidized nicotinamide adenine dinucleotide (NAD⁺) back to 1,3-DiPGA:



The net result of the enzymatic reactions [3] and [4] is the conversion of photosynthetically generated NADPH to NADH, which various NADH-requiring designer pathway enzymes such as NADH-dependent alcohol dehydrogenase 43 can use in producing butanol and related higher alcohols. When there is too much NADH, this NADPH/NADH conversion system can run also reversely to balance the supply of NADH and NADPH. Therefore, it is a preferred practice to innovatively utilize this NADPH/NADH conversion system under control of a designer switchable promoter such as nirA (or Nia1 for eukaryotic system) promoter when/if needed to achieve robust production of butanol and related higher alcohols. Various designer Calvin-cycle-channelled pathways in combination of a NADPH/NADH conversion mechanism with certain amino-acids-metabolism-related pathways for photobiological production of butanol and related higher alcohols are further described hereinbelow.

TABLE 1

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
03: Phosphoglycerate mutase (phosphoglyceromutase)	<i>Oceanithermus profundus</i> DSM 14977; ' <i>Nostoc azollae</i> ' 0708; <i>Thermotoga lettingae</i> TMO; <i>Syntrophothermus lipocalidus</i> DSM 12680; <i>Pelotomaculum thermopropionicum</i> SI; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Caldicellulosiruptor bescii</i> DSM 6725; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Thermotoga petrophila</i> RKU-1; <i>Deferribacter desulfuricans</i> SSM1; <i>Cyanobium</i> sp. PCC 7001; <i>Cyanothece</i> sp. PCC 8802; <i>Chlamydomonas reinhardtii</i> cytoplasm; <i>Aspergillus fumigatus</i> ; <i>Coccidioides immitis</i> ; <i>Leishmania braziliensis</i> ; <i>Ajellomyces capsulatus</i> ; <i>Monocercomonoides</i> sp.; <i>Aspergillus clavatus</i> ; <i>Arabidopsis thaliana</i> ; <i>Zea mays</i>	ADR35708; ADI65627, YP_003722750; YP_001470593, ABV33529; ADI02216, YP_003702781; YP_001212148; YP_001409891; YP_002573254, YP_002573195; ABS60234; ABQ47079, YP_001244998; YP_003496402, BAI80646; ZP_05046421; YP_003138980, YP_003138979; JGI Chlre2 protein ID 161689, GenBank: AF268078; XM_747847; XM_749597; XM_001248115; XM_001569263; XM_001539892; DQ665859; XM_001270940; NM_117020; M80912
04: Enolase	<i>Syntrophothermus lipocalidus</i> DSM 12680; ' <i>Nostoc azollae</i> ' 0708; <i>Thermotoga petrophila</i> RKU-1; <i>Spirochaeta thermophila</i> DSM 6192; <i>Cyanothece</i> sp. PCC 7822; <i>Hydrogenobacter thermophilus</i> TK-6; <i>Thermosynechococcus elongatus</i> BP-1, <i>Prochlorococcus marinus</i> str. MIT 9301; <i>Synechococcus</i> sp. WH 5701; <i>Trichodesmium erythraeum</i> IMS101; <i>Anabaena variabilis</i> ATCC 29413; <i>Nostoc</i> sp. PCC 7120; <i>Chlamydomonas reinhardtii</i> cytoplasm; <i>Arabidopsis thaliana</i> ; <i>Leishmania Mexicana</i> ; <i>Lodderomyces elongisporus</i> ; <i>Babesia bovis</i> ; <i>Sclerotinia sclerotiorum</i> ; <i>Pichia guilliermondii</i> ; <i>Spirotrichonympha leidyi</i> ; <i>Oryza sativa</i> ; <i>Trimastix pyriformis</i> ; <i>Leuconostoc mesenteroides</i> ; <i>Davidiella tassiana</i> ; <i>Aspergillus oryzae</i> ;	ADI02602, YP_003703167; ADI63801; ABQ46079; YP_003875216, ADN02943; YP_003886899, ADN13624; YP_003432637, BAI69436; BAC08209; ABO16851; ZP_01083626; ABG51970; ABA23124; BAB75237; GenBank: X66412, P31683; AK222035; DQ221745; XM_001528071; XM_001611873; XM_001594215; XM_001483612; AB221057; EF122486, U09450; DQ845796; AB088633; U82438; D64113; U13799; AY307449; U17973

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
05: Pyruvate kinase	<i>Schizosaccharomyces pombe</i> ; <i>Brassica napus</i> ; <i>Zea mays</i> <i>Syntrophothermus lipocalidus</i> DSM 12680; <i>Cyanothece</i> sp. PCC 8802; <i>Thermotoga lettingae</i> TMO; <i>Caldicellulosiruptor bescii</i> DSM 6725; <i>Geobacillus kaustophilus</i> HTA426; <i>Thermosynechococcus elongatus</i> BP-1; <i>Thermosiphon melanesiensis</i> B1429; <i>Thermotoga petrophila</i> RKU-1; <i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>Cyanothece</i> sp. PCC 7425; <i>Acaryochloris marina</i> MBIC11017; <i>Cyanothece</i> sp. PCC 8801; <i>Microcystis aeruginosa</i> NIES-843; <i>Cyanothece</i> sp. PCC 7822; <i>cyanobacterium</i> UCYN-A; <i>Arthrospira maxima</i> CS-328; <i>Synechococcus</i> sp. PCC 7335; <i>Chlamydomonas reinhardtii</i> cytoplasm; <i>Arabidopsis thaliana</i> ; <i>Saccharomyces cerevisiae</i> ; <i>Babesia bovis</i> ; <i>Sclerotinia sclerotiorum</i> ; <i>Trichomonas vaginalis</i> ; <i>Pichia guilliermondii</i> ; <i>Pichia stipitis</i> ; <i>Lodderomyces elongisporus</i> ; <i>Coccidioides immitis</i> ; <i>Trimastix pyriformis</i> ; <i>Glycine max</i> (soybean) <i>Peranema trichophorum</i> ; <i>Euglena gracilis</i>	ADI02459, YP_003703024; YP_002372431; YP_001471580, ABV34516; YP_002573139; YP_148872; NP_681306, BAC08068; YP_001306168, ABR30783; YP_001244312, ABQ46736; ABP67416, YP_001180607; ACL43749, YP_002482578; YP_001514814; YP_003138017; YP_001655408; YP_003890281; YP_003422225; ZP_03273505; ZP_05035056; JGI Chlre3 protein ID 138105; GenBank: AK229638; AY949876, AY949890, AY949888; XM_001612087; XM_001594710; XM_001329865; XM_001487289; XM_001384591; XM_001528210; XM_001240868; DQ845797; L08632 GenBank: EF114757; AB021127, AJ278425
06a: Pyruvate-NADP ⁺ oxidoreductase	<i>Mastigamoeba balamuthi</i> ; <i>Desulfovibrio africanus</i> ; <i>Entamoeba histolytica</i> ; <i>Trichomonas vaginalis</i> ; <i>Cryptosporidium parvum</i> ; <i>Cryptosporidium baileyi</i> ; <i>Giardia lamblia</i> ; <i>Entamoeba histolytica</i> ; <i>Hydrogenobacter thermophilus</i> ; <i>Clostridium pasteurianum</i> ; <i>Butyrivibrio fibrisolvens</i> ; butyrate-producing bacterium L2-50; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AY101767; Y09702; U30149; XM_001582310, XM_001313670, XM_001321286, XM_001307087, XM_001311860, XM_001314776, XM_001307250; EF030517; EF030516; XM_764947; XM_651927; AB042412; Y17727 GenBank: AB190764; DQ987697; Z92974;
06b: Pyruvate-ferredoxin oxidoreductase	<i>Clostridium beijerinckii</i> ; <i>Butyrivibrio fibrisolvens</i> ; <i>Ajellomyces capsulatus</i> ; <i>Aspergillus fumigatus</i> ; <i>Aspergillus clavatus</i> ; <i>Neosartorya fischeri</i> ; Butyrate-producing bacterium L2-50; <i>Arabidopsis thaliana</i> ; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AF494018; AB190764; XM_001537366; XM_741533; XM_001274776; XM_001262361; DQ987697; BT001208; Z92974;
07: Thiolase	<i>Clostridium beijerinckii</i> ; <i>Butyrivibrio fibrisolvens</i> ; Butyrate-producing bacterium L2-50; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AF494018; AB190764; DQ987697; Z92974
08: 3-Hydroxybutyryl-CoA dehydrogenase	<i>Clostridium beijerinckii</i> ; <i>Butyrivibrio fibrisolvens</i> ; Butyrate-producing bacterium L2-50; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AF494018; AB190764; DQ987697; Z92974
09: Crotonase	<i>Clostridium beijerinckii</i> ; <i>Butyrivibrio fibrisolvens</i> ; Butyrate-producing bacterium L2-50; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AF494018; AB190764; DQ987697; Z92974
10: Butyryl-CoA dehydrogenase	<i>Clostridium beijerinckii</i> ; <i>Butyrivibrio fibrisolvens</i> ; Butyrate-producing bacterium L2-50; <i>Thermoanaerobacterium thermosaccharolyticum</i> ;	GenBank: AY251646
11: Butyraldehyde dehydrogenase	<i>Geobacillus kaustophilus</i> HTA426; <i>Clostridium perfringens</i> str. 13; <i>Carboxydotherrmus hydrogenoformans</i> ; <i>Pseudovibrio</i> sp. JE062; <i>Clostridium carboxidivorans</i> P7; <i>Bacillus pseudofirmus</i> OF4; <i>Oceanobacillus theyensis</i> HTE831;	YP_148778, BAD77210; NP_561774, BAB80564; AAG23613; ZP_05082669, EEA96294; ADO12118; ADC48983, YP_003425875; NP_693981, BAC15015;
12a: NADH-dependent Butanol dehydrogenase		

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
12b: NADPH-dependent Butanol dehydrogenase	<i>Slackia exigua</i> ATCC 700122;	ZP_06159969, EEZ61452;
	<i>Fusobacterium ulcerans</i> ATCC 49185;	ZP_05633940;
	<i>Listeria monocytogenes</i> FSL J1-175;	ZP_05388801;
	<i>Chlorobium chlorochromatii</i> CaD3;	ABB28961;
	<i>Clostridium perfringens</i> D str. JGS1721;	ZP_02952811;
	<i>Clostridium perfringens</i> NCTC 8239;	ZP_02641897;
	<i>Clostridium perfringens</i> CPE str. F4969;	ZP_02638128;
	<i>Clostridium perfringens</i> B str. ATCC 3626;	ZP_02634798;
	<i>Clostridium botulinum</i> NCTC 2916;	EDT24774;
	<i>Nostoc</i> sp. PCC 7120;	ZP_02614964, ZP_02614746;
	<i>Clostridium perfringens</i> str. 13;	NP_488606, BAB76265;
	<i>Clostridium saccharobutylicum</i> ;	NP_562172, BAB80962;
	<i>Subdoligranulum variabile</i> DSM 15176;	AAA83520;
	<i>Butyrivibrio crossotus</i> DSM 2876;	EFB77036;
	<i>Oribacterium</i> sp. oral taxon 078 str. F0262;	EFF67629, ZP_05792927;
	<i>Clostridium</i> sp. M62/1;	ZP_06597730, EFE92592;
	<i>Clostridium hathewayi</i> DSM 13479;	EFE12215, ZP_06346636;
	<i>Subdoligranulum variabile</i> DSM 15176;	EFC98086, ZP_06115415;
	<i>Faecalibacterium prausnitzii</i> A2-165;	ZP_05979561;
	<i>Blautia hansenii</i> DSM 20583;	ZP_05615704, EEU95840;
	<i>Roseburia intestinalis</i> L1-82,	ZP_05853889, EEX22072;
	<i>Bacillus cereus</i> Rock3-28;	ZP_04745071, EEU99657;
	<i>Eubacterium rectale</i> ATCC 33656;	ZP_04236939, EEL31374;
	<i>Clostridium</i> sp. HGF2;	YP_002938098, ACR75964;
	<i>Atopobium rimae</i> ATCC 49626;	EFR36834;
	<i>Clostridium perfringens</i> D str. JGS1721;	ZP_03568088;
	<i>Clostridium perfringens</i> NCTC 8239;	ZP_02952006;
	<i>Clostridium butyricum</i> 5521;	ZP_02642725;
	<i>Clostridium carboxidivorans</i> P7;	ZP_02950013, ZP_02950012;
	<i>Clostridium botulinum</i> E3 str. Alaska E43;	ZP_06856327;
	<i>Clostridium novyi</i> NT;	YP_001922606, YP_001922335,
	<i>Clostridium botulinum</i> B str. Eklund 17B;	ACD52989; YP_878939;
	<i>Thermococcus</i> sp. AM4;	YP_001887401;
<i>Fusobacterium</i> sp. D11;	EEB74113;	
<i>Anaerococcus vaginalis</i> ATCC 51170;	EFD81183;	
<i>Clostridium perfringens</i> CPE str. F4969;	ZP_05473100, EEU12061;	
<i>Clostridium perfringens</i> B str. ATCC 3626;	EDT27639;	
13: Starch synthase	<i>Chlamydomonas reinhardtii</i> ;	EDT24389;
	<i>Phaseolus vulgaris</i> ;	GenBank: AF026422, AF026421,
	<i>Oryza sativa</i> ;	DQ019314, AF433156;
	<i>Arabidopsis thaliana</i> ;	AB293998; D16202, AB115917,
	<i>Colocasia esculenta</i> ;	AY299404; AF121673,
	<i>Amaranthus cruentus</i> ;	AK226881; NM_101044;
	<i>Parachlorella kessleri</i> ;	AY225862, AY142712;
	<i>Triticum aestivum</i> ;	DQ178026; AB232549; Y16340;
	<i>Sorghum bicolor</i> ;	AF168786; AF097922;
	<i>Astragalus membranaceus</i> ;	AF210699; AF019297; AF068834
14: Glucose-1-phosphate adenylyltransferase	<i>Perilla frutescens</i> ;	GenBank: NM_127730,
	<i>Zea mays</i> ;	NM_124205, NM_121927,
	<i>Ipomoea batatas</i>	AY059862; EF694839,
	<i>Chlamydomonas reinhardtii</i> ;	EF694838; AF087165; P55242;
	<i>Shigella flexneri</i> ;	NP_709206; T07674
15: Phosphoglucomutase	<i>Lycopersicon esculentum</i>	GenBank: AC105932, AF455812;
	<i>Oryza sativa</i> plastid;	XM_001536436; XM_001383281;
	<i>Ajellomyces capsulatus</i> ;	XM_001527445; XM_749345;
	<i>Pichia stipitis</i> ;	NM_124561, NM_180508,
	<i>Lodderomyces elongisporus</i> ;	AY128901; AY479974;
16: Hexose-phosphate-isomerase	<i>Aspergillus fumigatus</i> ;	AF455812; U89342, U89341
	<i>Arabidopsis thaliana</i> ;	YP_002633806, CAL27621;
17: Alpha-amylase;	<i>Populus tomentosa</i> ;	
	<i>Oryza sativa</i> ;	
	<i>Zea mays</i>	
	<i>Staphylococcus carnosus</i> subsp. <i>carnosus</i> TM300;	
Beta-amylase;	<i>Hordeum vulgare</i> aleuron cells;	GenBank: J04202;
	<i>Trichomonas vaginalis</i> ;	XM_001319100; EF143986;
	<i>Phanerochaete chrysosporium</i> ;	AY324649; NM_129551;
	<i>Chlamydomonas reinhardtii</i> ;	X07896;
	<i>Arabidopsis thaliana</i> ;	
	<i>Dictyoglomus thermophilum</i> heat-stable amylase gene;	
	<i>Arabidopsis thaliana</i> ;	GenBank: NM_113297; D21349;
	<i>Hordeum vulgare</i> ;	DQ166026;
	<i>Musa acuminata</i> ;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
Starch phosphorylase;	<i>Citrus</i> hybrid cultivar root; <i>Solanum tuberosum</i> chloroplast; <i>Arabidopsis thaliana</i> ; <i>Triticum aestivum</i> ; <i>Ipomoea batatas</i> ;	GenBank: AY098895; P53535; NM_113857, NM_114564; AF275551; M64362
18: Glucose-phosphate (glucose-6-phosphate) isomerase	<i>Chlamydomonas reinhardtii</i> ; <i>Saccharomyces cerevisiae</i> ; <i>Pichia stipitis</i> ; <i>Ajellomyces capsulatus</i> ; <i>Spinacia oleracea</i> cytosol; <i>Oryza sativa</i> cytoplasm; <i>Arabidopsis thaliana</i> ; <i>Zea mays</i>	JGI Chlre3 protein ID 135202; GenBank: M21696; XM_001385873; XM_001537043; T09154; P42862; NM_123638, NM_118595; U17225
19: Phosphofructose kinase	<i>Chlamydomonas reinhardtii</i> ; <i>Arabidopsis thaliana</i> ; <i>Ajellomyces capsulatus</i> ; <i>Yarrowia lipolytica</i> ; <i>Pichia stipitis</i> ; <i>Dictyostelium discoideum</i> ; <i>Tetrahymena thermophila</i> ; <i>Trypanosoma brucei</i> ; <i>Plasmodium falciparum</i> ; <i>Spinacia oleracea</i> ;	JGI Chlre2 protein ID 159495; GenBank: NM_001037043, NM_179694, NM_119066, NM_125551; XM_001537193; AY142710; XM_001382359, XM_001383014; XM_639070; XM_001017610; XM_838827; XM_001347929; DQ437575; GenBank: X69969; AF308587; NM_005165; XM_001609195; XM_001312327, XM_001312338; XM_001387466; NM_120057, NM_001036644
20: Fructose-diphosphate aldolase	<i>Chlamydomonas reinhardtii</i> chloroplast; <i>Fragaria x ananassa</i> cytoplasm; <i>Homo sapiens</i> ; <i>Babesia bovis</i> ; <i>Trichomonas vaginalis</i> ; <i>Pichia stipitis</i> ; <i>Arabidopsis thaliana</i>	GenBank: NM_127687, AF247559; AY742323; XM_001587391; AB240149; XM_001485684; DQ459379; AY742325; L36387; AY438596; U83414; EF575877;
21: Triose phosphate isomerase	<i>Arabidopsis thaliana</i> ; <i>Chlamydomonas reinhardtii</i> ; <i>Sclerotinia sclerotiorum</i> ; <i>Chlorella pyrenoidosa</i> ; <i>Pichia guilliermondii</i> ; <i>Euglena intermedia</i> ; <i>Euglena longa</i> ; <i>Spinacia oleracea</i> ; <i>Solanum chacoense</i> ; <i>Hordeum vulgare</i> ; <i>Oryza sativa</i>	GenBank: NM_127687, AF247559; AY742323; XM_001587391; AB240149; XM_001485684; DQ459379; AY742325; L36387; AY438596; U83414; EF575877;
34: NADPH-dependent Glyceraldehyde-3-phosphate dehydrogenase	<i>Staphylococcus aureus</i> 04-02981; <i>Staphylococcus lugdunensis</i> ; <i>Staphylococcus lugdunensis</i> HKU09; <i>Vibrio cholerae</i> BX 330286; <i>Vibrio</i> sp. Ex25; <i>Pseudomonas savastanoi</i> pv.; <i>Vibrio cholerae</i> B33; <i>Grimontia hollisae</i> CIP 101886; <i>Vibrio mimicus</i> MB-451; <i>Vibrio coralliilyticus</i> ATCC BAA-450; <i>Vibrio cholerae</i> MJ-1236; <i>Zea mays</i> cytosolic NADP dependent; <i>Apium graveolens</i> ; <i>Vibrio cholerae</i> B33; <i>Vibrio cholerae</i> TMA 21; <i>Vibrio cholerae</i> bv. <i>albensis</i> VL426; <i>Vibrio orientalis</i> CIP 102891; <i>Vibrio cholerae</i> MJ-1236; <i>Vibrio cholerae</i> CT 5369-93; <i>Vibrio</i> sp. RC586; <i>Vibrio furnissii</i> CIP 102972; <i>Vibrio metschnikovii</i> CIP 69.14; <i>Edwardsiella tarda</i> FL6-60; <i>Flavobacteriaceae bacterium</i> 3519-10; <i>Staphylococcus aureus</i> 04-02981; <i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> NCPPB 3335; <i>Vibrio cholerae</i> MJ-1236; <i>Streptococcus pyogenes</i> NZ131; <i>Helicobacter pylori</i> 908; <i>Streptococcus pyogenes</i> NZ131; <i>Staphylococcus lugdunensis</i> HKU09; <i>Vibrio</i> sp. Ex25; <i>Stenotrophomonas chelatiphaga</i> ; <i>Pseudoxanthomonas dokdonensis</i> ; <i>Stenotrophomonas maltophilia</i> ; <i>Vibrio cholerae</i> B33; <i>Photobacterium damsela</i> subsp. <i>damsela</i> CIP 102761; <i>Vibrio</i> sp. RC586; <i>Grimontia hollisae</i> CIP 101886;	ADC37857; ADC87332; YP_003471459; ZP_04395517; YP_003287699; ZP_07004478, EFI00105; ZP_04399616; ZP_06052988, EEY71738; ZP_06041160; ZP_05886203; YP_002876243; NP_001105589; AAF08296; EEO17521; EEO13209; EEO01829; ZP_05943395; ACQ62447; ZP_06049761; ZP_06079970; ZP_05878983; ZP_05883187; ADM41489; YP_003095198; ADC36961; ZP_07003925; ACQ61431, YP_002878104; YP_002285269; ADN80469; ACI60574; ADC88142; ACY51070; ADK67090; ADK67075; ADK67085, ACH90636; ZP_04401333; ZP_06155532; ZP_06080908; ZP_06052393;
35: NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase	<i>Staphylococcus aureus</i> 04-02981; <i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> NCPPB 3335; <i>Vibrio cholerae</i> MJ-1236; <i>Streptococcus pyogenes</i> NZ131; <i>Helicobacter pylori</i> 908; <i>Streptococcus pyogenes</i> NZ131; <i>Staphylococcus lugdunensis</i> HKU09; <i>Vibrio</i> sp. Ex25; <i>Stenotrophomonas chelatiphaga</i> ; <i>Pseudoxanthomonas dokdonensis</i> ; <i>Stenotrophomonas maltophilia</i> ; <i>Vibrio cholerae</i> B33; <i>Photobacterium damsela</i> subsp. <i>damsela</i> CIP 102761; <i>Vibrio</i> sp. RC586; <i>Grimontia hollisae</i> CIP 101886;	ADC36961; ZP_07003925; ACQ61431, YP_002878104; YP_002285269; ADN80469; ACI60574; ADC88142; ACY51070; ADK67090; ADK67075; ADK67085, ACH90636; ZP_04401333; ZP_06155532; ZP_06080908; ZP_06052393;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.			
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation	
36: (R)-Citramalate synthase (EC 2.3.1.182)	<i>Vibrio fumissii</i> CIP 102972;	EEX42220;	
	<i>Acidithiobacillus caldus</i> ATCC 51756;	ZP_05292346;	
	<i>Nostoc</i> sp. PCC 7120;	CAC41000;	
	<i>Vibrio cholerae</i> BX 330286;	EEO22474;	
	<i>Vibrio cholerae</i> TMA 21;	EEO13042;	
	<i>Nostoc</i> sp. PCC 7120;	CAC41000;	
	<i>Pinus sylvestris</i> ;	CAA04942;	
	<i>Cheilanthes yavapensis</i> ;	ACO58643, ACO58642;	
	<i>Cheilanthes wootonii</i> ;	ACO58624, ACO58623;	
	<i>Astrolepis laevis</i> ;	CBH41484, CBH41483;	
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003433013, ADO45737,	
	<i>Geobacter bemidjensis</i> Bem;	BAI69812;	
	<i>Geobacter sulfurreducens</i> KN400;	ACH38284;	
	<i>Methanobrevibacter ruminantium</i> M1;	ADI84633;	
	<i>Leptospira biflexa</i> serovar Patoc strain 'Patoc 1 (Paris)';	CP001719;	
	<i>Leptospira biflexa</i> serovar Monteralero;	ABK13757;	
	<i>Leptospira interrogans</i> serovar Australis;	ABK13756;	
	<i>Leptospira interrogans</i> serovar Pomona;	ABK13755;	
	<i>Leptospira interrogans</i> serovar Autumnalis;	ABK13753;	
	<i>Leptospira interrogans</i> serovar Pyrogenes;	ABK13754;	
	<i>Leptospira interrogans</i> serovar Canicola;	ABK13752;	
	<i>Leptospira interrogans</i> serovar Lai;	ABK13751;	
	<i>Acetohalobium arabaticum</i> DSM 5501;	ABK13750;	
	<i>Leadbetterella byssophila</i> DSM 17132;	ABK13749;	
	<i>Bacteroides xylanisolvens</i> XB1A;	ADL11763,	
	<i>Mucilaginibacter paludis</i> DSM 18603;	YP_003998693;	
	<i>Prevotella ruminicola</i> 23;	CBK66631;	
	<i>Flavobacterium johnsoniae</i> UW101;	EFQ72644;	
<i>Victivallis vadensis</i> ATCC BAA-548;	ADE82919;		
<i>Prevotella copri</i> DSM 18205;	ABQ04337;		
<i>Alistipes shahii</i> WAL 8301;	ZP_06244204,		
<i>Methylobacter tundripaludum</i> SV96;	EFA99692;		
<i>Methanosarcina mazei</i> Go1;	EFB36404, ZP_06251228;		
37: (R)-2-Methylmalate dehydratase (large and small subunits) (EC 4.2.1.35)	<i>Eubacterium eligens</i> ATCC 27750	CBK64953;	
	<i>Methanocaldococcus jamaerschii</i> ;	ZP_07654184;	
	<i>Sebaldella termitidis</i> ATCC 33386;	NP_632695;	
	<i>Eubacterium eligens</i> ATCC 27750;	YP_002930810, YP_002930809;	
	38: 3-Isopropylmalate dehydratase (large + small subunits) (EC 4.2.1.33)	<i>Thermotoga petrophila</i> RKU-1;	P81291;
		<i>Cyanothece</i> sp. PCC 7822;	ACZ06998;
		<i>Syntrophothermus lipocalidus</i> DSM 12680;	ACR72362, ACR72361,
		<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903;	ACR72363, YP_002930808;
		<i>Pelotomaculum thermopropionicum</i> SI,	ABQ46641, ABQ46640;
		<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_003886427, YP_003889452;
		<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903;	ADI02900, ADI02899,
		<i>E. coli</i> ;	YP_003703465, ADI01294;
		<i>Spirochaeta thermophila</i> DSM 6192;	ABP66933, ABP66934;
		<i>Pelotomaculum thermopropionicum</i> SI;	YP_001211082, YP_001211083;
		<i>Hydrogenobacter thermophilus</i> TK-6;	YP_002573950, YP_002573949;
		<i>Deferribacter desulfuricans</i> SSM1;	YP_001180124, YP_001180125;
		<i>Anoxybacillus flavithermus</i> WK1;	leuC, ECK0074, JW0071;
		<i>Thermosynechococcus elongatus</i> BP-1;	leuD, ECK0073, JW0070;
		<i>Geobacillus kaustophilus</i> HTA426;	YP_003875294, YP_003873373;
		<i>Synechocystis</i> sp. PCC 6803;	YP_001213069, YP_001213068;
		<i>Chlamydomonas reinhardtii</i> ;	YP_003433547, YP_003432351;
		<i>Thermotoga petrophila</i> RKU-1;	YP_003495505, YP_003495504;
		<i>Cyanothece</i> sp. PCC 7822;	ACJ32977, ACJ32978;
		<i>Thermosynechococcus elongatus</i> BP-1;	BAC08461, BAC08786;
		<i>Syntrophothermus lipocalidus</i> DSM 12680;	BAD76941, BAD76940;
		<i>Caldicellulosiruptor bescii</i> DSM 6725;	BAA18738, BAA18298;
		<i>Paludibacter propionigenes</i> WB4;	XP_001702135, XP_001696402;
		<i>Leadbetterella byssophila</i> DSM 17132;	ABQ46392, YP_001243968;
<i>Caldicellulosiruptor saccharolyticus</i>		YP_003888480, ADN15205;	
		BAC09152, NP_682390;	
		ADI02898, YP_003703463;	
		ADQ78220;	
	YP_002573948;		
	YP_003998692;		
	ABP66935;		

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
40: 2-Isopropylmalate synthase (EC 2.3.3.13)	DSM 8903; <i>Thermus thermophilus</i> ;	AAA16706, YP_001180126;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001211084;
	<i>Geobacillus kaustophilus</i> HTA426;	YP_148510, BAD76942;
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003433176;
	<i>Spirochaeta thermophila</i> DSM 6192;	YP_003873639;
	<i>Deferribacter desulfuricans</i> SSM1;	YP_003495917;
	<i>Anoxybacillus flavithermus</i> WK1;	YP_002314961;
	<i>Volvox carteri</i> f. nagariensis;	XP_002955062, EFJ43816;
	<i>Chlamydomonas reinhardtii</i> ;	XP_001701074, XP_001701073;
	<i>Ostreococcus tauri</i> ;	XP_003083133;
	<i>Thermotoga petrophila</i> RKU-1;	ABQ46395, YP_001243971;
	<i>Cyanothece</i> sp. PCC 7822;	YP_003890122, ADN16847;
	<i>Cyanothece</i> sp. PCC 8802;	ACU99797;
	<i>Nostoc punctiforme</i> PCC 73102;	ACC82459;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001211081;
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003432474, BAI69273;
	<i>E. coli</i> ; <i>Caldicellulosiruptor</i>	NP_414616, AAC73185;
	<i>saccharolyticus</i> DSM 8903;	ABP66753, YP_001179944;
	<i>Syntrophothermus lipocalidus</i> DSM	YP_003703466, ADI02901;
	12680; <i>Geobacillus kaustophilus</i>	YP_148511, BAD76943;
	HTA426; <i>Caldicellulosiruptor bescii</i>	YP_002572404;
	DSM 6725; <i>Anoxybacillus flavithermus</i>	YP_002314960, ACJ32975;
	WK1; <i>Deferribacter desulfuricans</i>	YP_003496874, BAI811118;
SSM1; <i>Thermosynechococcus elongatus</i>	NP_682187, BAC08949;	
BP-1; <i>Spirochaeta thermophila</i> DSM	ADN03009, YP_003875282;	
6192; <i>Thermotoga lettingae</i> TMO;	YP_001469896, ABV32832;	
<i>Volvox carteri</i> f. nagariensis;	XP_002945733,	
<i>Micromonas</i> sp. RCC299;	EFJ52728;	
<i>Micromonas pusilla</i> CCMP1545;	ACO69978, XP_002508720;	
<i>Chlamydomonas reinhardtii</i> ;	XP_003063010, EEH52949;	
41: isopropylmalate isomerase large/small subunits (EC 4.2.1.33)	<i>Geobacillus kaustophilus</i> HTA426;	XP_001696603, EDP08580;
	<i>Anabaena variabilis</i> ATCC 29413;	YP_148509, YP_148508;
	<i>Synechocystis</i> sp. PCC 6803;	YP_324467, YP_324466;
	<i>Anoxybacillus flavithermus</i> WK1;	NP_442926, NP_441618;
	<i>Thermosynechococcus elongatus</i> BP-1;	YP_002314962, YP_002314963;
	<i>Spirochaeta thermophila</i> DSM 6192;	NP_682024, NP_681699;
	<i>Salmonella enterica</i> subsp. <i>enterica</i>	YP_003873372;
	serovar <i>Typhimurium</i> str. D23580;	CBG23133, CBG23132;
	<i>Staphylococcus aureus</i> A5937;	ZP_05702396;
	<i>Francisella philomiragia</i> subsp.	EET20545;
	<i>philomiragia</i> ATCC 25015;	AAA53236;
	<i>Neisseria lactamica</i> ; <i>Francisella</i>	ABK88972;
	<i>novicida</i> U112; <i>Staphylococcus aureus</i>	EEV86047;
	A5937; <i>Staphylococcus aureus</i> subsp.	ZP_05607839;
	<i>aureus</i> 68-397; <i>Fusobacterium</i> sp.	EEO38992;
	2_1_31; <i>Francisella novicida</i> GA99-	EDN35429;
	3549; marine <i>bacterium</i> HP15;	ADP98363, ADP98362;
	<i>Bacillus licheniformis</i> ATCC 14580;	YP_092517, YP_092516;
	<i>Rhodobacter sphaeroides</i> 2.4.1;	YP_353947, YP_353945;
	<i>Bordetella petrii</i> DSM 12804;	YP_001631647, YP_001631646;
	<i>Agrobacterium vitis</i> S4;	YP_002551071, YP_002551071;
	<i>Lactococcus lactis</i> ;	AAS49166;
	<i>Lactococcus lactis</i> subsp. <i>lactis</i> KF147;	ADA65057, YP_003353820;
<i>Lactococcus lactis</i> subsp. <i>Lactis</i> ;		
<i>Kluyveromyces marxianus</i> ;	CAG34226;	
<i>Kluyveromyces lactis</i> ;	AAA35267;	
<i>Mycobacterium avium</i> 104;	CAA59953;	
<i>Mycobacterium ulcerans</i> Agy99;	A0QBE6;	
<i>Mycobacterium bovis</i> ;	A0PL16;	
<i>Mycobacterium leprae</i> ;	Q7U140;	
<i>Proteus mirabilis</i> HI4320;	Q9CBD6;	
<i>Staphylococcus aureus</i> 04-02981;	YP_002150004;	
<i>Acetobacter pasteurianus</i> ;	ADC36400;	
<i>Saccharomyces cerevisiae</i> ;	AAM21208;	
<i>Zymomonas mobilis</i> subsp. <i>mobilis</i> CP4;	CAA39398;	
<i>Mycobacterium tuberculosis</i> ;	AAA27696;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
43: Alcohol dehydrogenase (NAD dependent) (EC 1.1.1.1);	<i>Mycobacterium smegmatis</i> str. MC2 155; <i>Mycobacterium bovis</i> BCG str. Pasteur 1173P2;	O53865; A0R480; A1KGY5;
	<i>Thermoplasma volcanium</i> GSS1;	BAB59540
	<i>Gluconacetobacter hansenii</i> ATCC 23769; <i>Saccharomyces cerevisiae</i> ;	ZP_06834544; CAA89136;
	<i>Aeropyrum pernix</i> K1;	NP_148480;
	<i>Rhodobacteriales bacterium</i> HTCC2083;	ZP_05073895;
	<i>Bradyrhizobium japonicum</i> USDA 110;	NP_769420;
	<i>Syntrophothermus lipocalidus</i> DSM 12680; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Desulfotalea psychrophila</i> LSv54; <i>Acetobacter pasteurianus</i> IFO 3283-03; <i>Gluconobacter oxydans</i> 621H; <i>Aeromonas hydrophila</i> subsp. <i>hydrophila</i> ATCC 7966; <i>Acetobacter pasteurianus</i> IFO 3283-01; <i>Streptomyces hygrosopicus</i> ATCC 53653;	ADJ01021; YP_001411173; YP_065604; BAI03878; YP_192500; ABK38651; BAI00830; EFL29096;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001211038, BAF58669;
	<i>Fusobacterium</i> sp. 7_1;	ZP_04573952, EEO43462;
	<i>Pichia pastoris</i> GS115;	XP_002494014, XP_002490014;
	<i>Pichia pastoris</i> GS115;	CAY71835, XP_002492217,
	<i>Escherichia coli</i> str. K-12 substr. MG1655;	CAY67733;
	<i>Clostridium hathewayi</i> DSM 13479;	yqhD, NP_417484, AAC76047;
44: Alcohol dehydrogenase (NADPH dependent) (EC 1.1.1.2);	<i>Clostridium butyricum</i> 5521;	EFC99049;
	<i>Fusobacterium ulcerans</i> ATCC 49185;	ZP_02948287
	<i>Fusobacterium</i> sp. D11; <i>Desulfovibrio desulfuricans</i> subsp. <i>desulfuricans</i> str. G20; <i>Clostridium novyi</i> NT;	ZP_05632371; ZP_05440863;
	<i>Clostridium tetani</i> E88;	YP_389756;
	<i>Aureobasidium pullulans</i> ;	YP_878957;
	<i>Scheffersomyces stipitis</i> CBS 6054,	NP_782735;
	<i>Thermotoga lettingae</i> TMO;	ADG56699;
	<i>Thermotoga petrophila</i> RKU-1;	ABN66271, XP_001384300;
	<i>Coprinopsis cinerea</i> okayama7#130;	YP_001471424;
	<i>Saccharomyces cerevisiae</i> EC1118;	YP_001244106;
	<i>Saccharomyces cerevisiae</i> JAY291;	XP_001834460;
	<i>Thermaerobacter subterraneus</i> DSM 13965; <i>Cyanothece</i> sp. PCC 7822;	CAY82157;
	<i>Thermus</i> sp.; <i>Rhodothermus marinus</i> ;	EEU07174;
	<i>Thermosynechococcus elongatus</i> BP-1;	EFR61439;
	<i>Leadbetterella byssophila</i> DSM 17132;	YP_003887888;
	<i>Riemerella anatipestifer</i> DSM 15868;	BAA07723; CAA67760;
	<i>Mucilaginibacter paludis</i> DSM 18603;	NP_682702, BAC09464;
	<i>Truepera radiovictrix</i> DSM 17093;	YP_003998059, ADQ17706;
	<i>Ferrimonas balearica</i> DSM 9799;	ADQ81501, YP_004045007;
	<i>Meiothermus silvanus</i> DSM 9946;	EFQ77722;
	<i>Nocardioopsis dassonvillei</i> subsp. <i>dassonvillei</i> DSM 43111; <i>E. coli</i> ,	YP_003706036;
	<i>Meiothermus ruber</i> DSM 1279;	YP_003911597, ADN74523;
	<i>Olsenella uli</i> DSM 7084;	YP_003685046;
<i>Kiedonobacter racemifer</i> DSM 44963;	YP_003681843;	
<i>Rhodopirellula baltica</i> SH 1;	ZP_07594313, ZP_07565817;	
<i>Oceanithermus profundus</i> DSM 14977;	ADD27759;	
<i>marine bacterium</i> HP15;	YP_003801346, ADK68466;	
<i>Marivirga tractuosa</i> DSM 4126;	ZP_06967036, EFH90147;	
<i>Mucilaginibacter paludis</i> DSM 18603;	NP_866412, CAD78193;	
<i>Streptomyces coelicolor</i> A3(2);	ADR36285;	
<i>Delftia acidovorans</i> SPH-1;	ADP96559;	
<i>Actinobacillus pleuropneumoniae</i> serovar 13 str. N273; <i>Prochlorococcus marinus</i> str. MIT 9301;	ADR23252;	
<i>Prochlorococcus marinus</i> str. NATL1A	ZP_07746438;	
<i>Prochlorococcus marinus</i> str. MIT 9515; <i>Clostridium cellulovorans</i> 743B;	NP_627344;	
<i>Neisseria meningitidis</i> Z2491;	ABX34873;	
<i>Deinococcus geothermalis</i> DSM 11300;	ZP_07544559;	
<i>Micromonospora</i> sp. L5;	ABO18389;	
	ABM76577;	
	ABM72969;	
	YP_003842669, ADL50905;	
	CAM07667;	
	ABF44963;	
	ZP_06399624;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
46: Aspartate aminotransferase (EC 2.6.1.1)	<i>Chlorobium phaeobacteroides</i> DSM 266; <i>Arthrobacter</i> sp. FB24;	ABL64615; YP_830113;
	<i>Rhodomicrobium vannielii</i> ATCC 17100; <i>Gordonia bronchialis</i> DSM 43247; <i>Thermus aquaticus</i> Y51MC23;	YP_004010507; YP_003273502; ZP_03496338;
	<i>Burkholderia ambifaria</i> IOP40-10; <i>Thermotoga lettingae</i> TMO;	ZP_02894226; YP_001470126;
	<i>Synechococcus elongatus</i> PCC 6301; <i>Synechococcus elongatus</i> PCC 7942;	YP_172275; YP_401562;
	<i>Thermosiphon melanesiensis</i> BI429; <i>Thermotoga petrophila</i> RKU-1;	YP_001306480; YP_001244588;
	<i>Thermus thermophilus</i> ;	BAA07487;
	<i>Anoxybacillus flavithermus</i> WK1;	YP_002315494;
	<i>Bacillus</i> sp.; <i>E. coli</i> ,	AAA22250; aspC: BAB34434;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001211971;
	<i>Phormidium lapideum</i> ;	BAB86290;
	<i>Fervidobacterium nodosum</i> Rt17-B1;	YP_001410686, YP_001409589;
	<i>Geobacillus kaustophilus</i> HTA426;	YP_148025, YP_147632,
	<i>Thermosynechococcus elongatus</i> BP-1;	YP_146225; NP_683147;
	<i>Anoxybacillus flavithermus</i> WK1;	ACI34747;
	<i>Geobacillus kaustophilus</i> HTA426;	BAD77213, BAD76064;
	<i>Spirochaeta thermophila</i> DSM 6192;	YP_003874653;
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_002572445;
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903;	YP_001179582;
	<i>Arabidopsis thaliana</i> ;	AAA79371;
	<i>Glycine max</i> ;	AAA33942;
	<i>Lupinus angustifolius</i> ;	CAA42430;
	<i>Chlamydomonas reinhardtii</i> ;	XP_001696609;
	<i>Micromonas pusilla</i> CCMP1545;	XP_003060871;
	<i>Thermotoga lettingae</i> TMO;	YP_001470361, ABV33297;
	<i>Cyanothece</i> sp. PCC 8802;	YP_003136939;
	<i>Thermotoga petrophila</i> RKU-1	YP_001244864, YP_001243977;
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003432105, BAI68904;
	<i>Anoxybacillus flavithermus</i> WK1;	ACI35001;
	<i>Bacillus</i> sp.;	AAA22251;
	<i>Spirochaeta thermophila</i> DSM 6192;	YP_003873788, ADN01515;
	<i>Anoxybacillus flavithermus</i> WK1;	ACI34043, YP_002316986;
	<i>Geobacillus kaustophilus</i> HTA426;	BAD77480, YP_149048;
	<i>Syntrophothermus lipocalidus</i> DSM 12680; <i>E. coli</i> ;	ADI02230, YP_003702795;
	<i>Thermosynechococcus elongatus</i> BP-1;	ZP_07594328, ZP_07565832;
	<i>Fervidobacterium nodosum</i> Rt17-B1;	NP_682623, BAC09385;
	<i>Spirochaeta thermophila</i> DSM 6192;	ABS59942, YP_001410786;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_003873302, ADN01029;
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>Caldicellulosiruptor bescii</i> DSM 6725; <i>Thermosiphon melanesiensis</i> BI429; <i>Thermotoga lettingae</i> TMO;	YP_001212149, YP_001211837;
	<i>Arabidopsis thaliana</i> ;	ABP66605;
	<i>Chlamydomonas reinhardtii</i> ;	YP_002573821;
		YP_001307097, ABR31712;
		YP_001470985, ABV33921;
		CAA67376;
		XP_001698576, EDP08069,
		XP_001695256;
		YP_001470981, ABV33917;
	ABG50031;	
	ABM76828;	
	ABQ47283, YP_001244859;	
	ABP67176, YP_001180367;	
	ADI01804, YP_003702369;	
	YP_001460230, YP_001464895;	
	YP_001409594, ABS59937;	
	YP_002573009;	
	YP_001307092, ABR31707;	
	YP_003875128, ADN02855;	
	YP_001211836, BAF59467;	
	YP_003432252, BAI69051;	
	YP_002316029, ACJ34044;	
	YP_147128, BAD75560;	
	YP_003496635, BAI80879;	
	NP_680860, BAC07622;	
	AAG23574, AAG23573;	
48: Aspartate-semialdehyde dehydrogenase	<i>Thermotoga lettingae</i> TMO;	YP_001470981, ABV33917;
	<i>Trichodesmium erythraeum</i> IMS101;	ABG50031;
	<i>Prochlorococcus marinus</i> str. MIT 9303;	ABM76828;
	<i>Thermotoga petrophila</i> RKU-1;	ABQ47283, YP_001244859;
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>Syntrophothermus lipocalidus</i> DSM 12680; <i>E. coli</i> ;	ABP67176, YP_001180367;
	<i>Fervidobacterium nodosum</i> Rt17-B1	ADI01804, YP_003702369;
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_001460230, YP_001464895;
	<i>Thermosiphon melanesiensis</i> BI429;	YP_001409594, ABS59937;
	<i>Spirochaeta thermophila</i> DSM 6192;	YP_002573009;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001307092, ABR31707;
<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003875128, ADN02855;	
<i>Anoxybacillus flavithermus</i> WK1;	YP_001211836, BAF59467;	
<i>Geobacillus kaustophilus</i> HTA426;	YP_003432252, BAI69051;	
<i>Deferribacter desulfuricans</i> SSM1;	YP_002316029, ACJ34044;	
<i>Thermosynechococcus elongatus</i> BP-1;	YP_147128, BAD75560;	
	YP_003496635, BAI80879;	
	NP_680860, BAC07622;	
	AAG23574, AAG23573;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
49: Homoserine dehydrogenase	<i>Carboxydotherrnus hydrogenoformans</i> ;	XP_001695059, EDP02211;
	<i>Chlamydomonas reinhardtii</i> ;	ABH11018;
	<i>Polytomella parva</i> ;	ACU30050;
	<i>Glycine max</i> ;	ACG41594;
	<i>Zea mays</i> ;	ABR26065;
	<i>Oryza sativa</i> Indica Group;	
	<i>Syntrophothermus lipocalidus</i> DSM 12680; <i>Cyanothece</i> sp. PCC 7822;	ADI02231, YP_003702796;
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_003887242;
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>E. coli</i> ;	YP_002573819;
	<i>Spirochaeta thermophila</i> DSM 6192;	ABP66607, YP_001179798;
	<i>Pelotomaculum thermopropionicum</i> SI;	EFJ98002;
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003873441, ADN01168;
	<i>Anoxybacillus flavithermus</i> WK1;	YP_001212151, BAF59782;
	<i>Geobacillus kaustophilus</i> HTA426;	YP_003431981, BAI68780;
<i>Deferribacter desulfuricans</i> SSM1;	YP_002316756, ACJ34771;	
<i>Thermosynechococcus elongatus</i> BP-1;	YP_148817, BAD77249;	
<i>Glycine max</i> ;	YP_003496401, BAI80645;	
<i>Chlamydomonas reinhardtii</i> ;	NP_681068, BAC07830;	
<i>Micromonas</i> sp. RCC299;	ABG78600, AAZ98830;	
<i>Thermotoga petrophila</i> RKU-1;	XP_001699712, EDP07408;	
<i>Cyanothece</i> sp. PCC 7822;	ACO69662, XP_002508404;	
<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_001243979, ABQ46403;	
<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>E. coli</i> ;	YP_003886645;	
<i>Anoxybacillus flavithermus</i> WK1;	YP_002573820;	
<i>Geobacillus kaustophilus</i> HTA426;	ABP66606, YP_001179797;	
<i>Thermosynechococcus elongatus</i> BP-1;	AP_000667, BAB96580;	
<i>Pelotomaculum thermopropionicum</i> SI;	YP_002316754, ACJ34769;	
<i>Hydrogenobacter thermophilus</i> TK-6;	YP_148815, BAD77247;	
<i>Chlamydomonas reinhardtii</i> ;	NP_682555, BAC09317;	
<i>Prototheca wickerhamii</i> ;	YP_001212150, BAF59781;	
<i>Arabidopsis thaliana</i> ;	YP_003433124, BAI69923;	
<i>Glycine max</i> ;	XP_001701899, EDP06874;	
<i>Zea mays</i> ;	ABC24954;	
<i>Thermotoga petrophila</i> RKU-1;	NP_179318, AAD33097;	
<i>Cyanothece</i> sp. PCC 7425;	ACU26535;	
<i>Thermosiphon melanesiensis</i> BI429;	ACG46592;	
<i>Syntrophothermus lipocalidus</i> DSM 12680; <i>E. coli</i> ;	YP_001243978, ABQ46402;	
<i>Pelotomaculum thermopropionicum</i> SI;	YP_002485009;	
<i>Anoxybacillus flavithermus</i> WK1;	YP_001306558, ABR31173;	
<i>Caldicellulosiruptor bescii</i> DSM 6725;	ADI02519, YP_003703084;	
<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>Hydrogenobacter thermophilus</i> TK-6; <i>Geobacillus kaustophilus</i> HTA426;	AP_000668, NP_414545;	
<i>Thermosynechococcus elongatus</i> BP-1;	YP_001213220;	
<i>Spirochaeta thermophila</i> DSM 6192;	YP_002316755, ACJ34770;	
<i>Deferribacter desulfuricans</i> SSM1;	YP_002572552;	
<i>Geobacillus kaustophilus</i> HTA426;	YP_001180015, ABP66824;	
<i>Prochlorococcus marinus</i> str. MIT 9202; <i>Synechococcus</i> sp. PCC 7335;	YP_003433070, YP_003433019,	
<i>Thermotoga petrophila</i> RKU-1;	BAI69869, BAI69818;	
<i>Pelotomaculum thermopropionicum</i> SI;	YP_148816, YP_147614;	
<i>Anoxybacillus flavithermus</i> WK1;	NP_682017, NP_681772,	
<i>Deferribacter desulfuricans</i> SSM1;	BAC08534, BAC08779;	
<i>E. coli</i> ;	YP_003873303, ADN01030;	
<i>Neisseria lactamica</i> ATCC 23970;	YP_003495358, BAI79602;	
<i>Citrobacter youngae</i> ATCC 29220;	BAD76058, BAD75876,	
<i>Neisseria polysaccharea</i> ATCC 43768;	YP_147626, YP_147444;	
<i>Providencia rettgeri</i> DSM 1131;	ZP_05137562; ZP_05035047;	
<i>Neisseria subflava</i> NJ9703;	ABQ46585, YP_001244161;	
<i>Mannheimia haemolytica</i> PHL213;	YP_001210652, BAF58283;	
<i>Achromobacter piechaudii</i> ATCC 43553; <i>Neisseria meningitidis</i> ATCC 13091; <i>Synechococcus</i> sp. CC9902;	YP_002315804, YP_002315746;	
<i>Synechococcus</i> sp. PCC 7002;	YP_003497384, BAI81628;	
	YP_001746093, ZP_07690697;	
	EEZ76650, ZP_05986317;	
	EFE07783, ZP_06571237;	
	EFH23894, ZP_06863451;	
	EFE52186, ZP_06127162;	
	EFC51529, ZP_05985502;	
	ZP_04978734;	
	ZP_06687730, ZP_06684811;	
	ZP_07369980, EFM04207;	
	ABB26032;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
53: Acetolactate synthase (EC 2.2.1.6)	<i>Synechococcus</i> sp. WH 8109;	ACA99606;
	<i>Cyanobium</i> sp. PCC 7001;	ZP_05790446, EEX07646;
	<i>Anabaena variabilis</i> ATCC 29413;	EDY39077, ZP_05045768;
	<i>Microcoleus chthonoplastes</i> PCC 7420;	ABA20300;
	<i>Chlamydomonas reinhardtii</i> ;	ZP_05029756;
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903;	XP_001701816, EDP06791; ABP66750, ABP66751, YP_001179942, ABP66455, YP_001179941, YP_001179646;
	<i>Thermotoga petrophila</i> RKU-1;	YP_001243976, YP_003345845, ADA66432, ADA66431, ABQ46399, YP_001243975, ABQ46400, YP_003345846;
	<i>Thermosynechococcus elongatus</i> BP-1;	NP_682614, BAC09376, NP_681670, BAC08432, NP_682086;
	<i>Syntrophothermus lipocalidus</i> DSM 12680;	ADI02904, YP_003703469, ADI02903, YP_003703468;
	<i>Pelotomaculum thermopropionicum</i> SI;	BAF58709, BAF58917, YP_001211286, YP_001211078;
	<i>Geobacillus kaustophilus</i> HTA426;	BAD76946, YP_148514, BAD76945, YP_148513;
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	ACM59790, ACM59628, ACM59629, YP_002572563, YP_002572401, YP_002572402; YP_003432299, YP_003432300,
	<i>Hydrogenobacter thermophilus</i> TK-6;	BAI69099, BAI69098; YP_003874926, YP_003874927,
	<i>Spirochaeta thermophila</i> DSM 6192;	ADN02654, ADN02653, ACJ33615, YP_002314957,
	<i>Anoxybacillus flavithermus</i> WK1;	ACJ32972, ACJ32973, YP_002314958;
	<i>Deferribacter desulfuricans</i> SSM1;	YP_003496879, BAI81123, YP_003496878, BAI81122; AP_004121, BAE77622,
	<i>Escherichia coli</i> str. K-12 substr. W3110;	AP_004122, BAE77623, BAE77528, AP_004027, BAB96646, AP_000741; BAA12700;
	<i>Saccharomyces cerevisiae</i> ,	EDN64495, CAA89744, EDV09697;
	<i>Thermus aquaticus</i> ;	YP_001735999, ACB00744;
	<i>Synechococcus</i> sp. PCC 7002;	YP_002376012;
	<i>Cyanothece</i> sp. PCC 7424;	YP_324035;
	<i>Anabaena variabilis</i> ATCC 29413;	NP_487595, BAB75254;
	<i>Nostoc</i> sp. PCC 7120;	YP_001655615;
<i>Microcystis aeruginosa</i> NIES-843;	NP_441297, BAA17984,	
<i>Synechocystis</i> sp. PCC 6803;	CAA66718, NP_441304, NP_442206, BAA10276 ; YP_478353;	
<i>Synechococcus</i> sp. JA-2-3B'a(2-13);	YP_475372, ABD00213, ABD00270, YP_475476, YP_475533;	
<i>Synechococcus</i> sp. JA-3-3Ab;	AAC03784, AAB88292, XP_001700185, EDO98300, XP_001695168, EDP01876;	
<i>Chlamydomonas reinhardtii</i> ;	AAC04854, AAB88296; CAB07802 (AlsS); AAU42663 (AlsS); ADI02902, YP_003703467;	
<i>Volvox carteri</i> ;	ABP66752, YP_001179943; AAA67577, YP_001460567;	
<i>Bacillus subtilis</i> subsp. <i>subtilis</i> str. 168;	ABQ46398, YP_001243974; YP_004050904;	
<i>Bacillus licheniformis</i> ATCC 14580;	YP_003874858, ADN02585;	
<i>Syntrophothermus lipocalidus</i> DSM 12680; <i>Caldicellulosiruptor</i> <i>saccharolyticus</i> DSM 8903; <i>E. coli</i> ;	YP_001211079, BAF58710; YP_003885458;	
<i>Thermotoga petrophila</i> RKU-1;	YP_003433279, BAI70078;	
<i>Calditerrivibrio nitroreducens</i> DSM 19672;	YP_002314959, ACJ32974;	
<i>Spirochaeta thermophila</i> DSM 6192;		
<i>Pelotomaculum thermopropionicum</i> SI;		
<i>Cyanothece</i> sp. PCC 7822;		
<i>Hydrogenobacter thermophilus</i> TK-6;		
<i>Anoxybacillus flavithermus</i> WK1;		

54:
Ketol-acid reductoisomerase
(EC 1.1.1.86)

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.				
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation		
55: Dihydroxy-acid dehydratase (EC 4.2.1.9)	<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_002572403;		
	<i>Geobacillus kaustophilus</i> HTA426;	YP_148512, BAD76944;		
	<i>Deferribacter desulfuricans</i> SSM1;	YP_003496877, BAI81121;		
	<i>Thermosynechococcus elongatus</i> BP-1;	NP_683044, BAC09806;		
	<i>Cyanothece</i> sp. PCC 7425;	YP_002482078;		
	<i>Nostoc punctiforme</i> PCC 73102;	ACC82013;		
	<i>Trichodesmium erythraeum</i> IMS101;	ABG53327;		
	<i>Synechococcus</i> sp. PCC 7335;	ZP_05036558;		
	<i>Microcoleus chthonoplastes</i> PCC 7420;	ZP_05026584;		
	<i>Prochlorococcus marinus</i> str. MIT 9301; <i>Cyanobium</i> sp. PCC 7001;	ABO18124;		
	<i>Arthrospira</i> sp. PCC 8005;	EDY39000;		
	<i>Arabidopsis thaliana</i> ;	ZP_07166132;		
	<i>Pisum sativum</i> (pea);	CAA48253, NP_001078309;		
	<i>Zea mays</i> ;	CAA76854;		
	<i>Chlamydomonas reinhardtii</i> ;	ACG35752;		
	<i>Polytomella parva</i> ;	XP_001702649, EDP06428;		
	<i>Thermotoga petrophila</i> RKU-1;	ABH11013;		
	<i>Cyanothece</i> sp. PCC 7822;	YP_001243973, ABQ46397;		
	<i>Marivirga tractuosa</i> DSM 4126;	YP_003887466;		
	<i>Geobacillus kaustophilus</i> HTA426;	YP_004053736;		
	<i>Syntrophothermus lipocalidus</i> DSM 12680;	YP_147899, BAD76331,		
	<i>Spirochaeta thermophila</i> DSM 6192;	YP_147822, BAD76254;		
	<i>Anoxybacillus flavithermus</i> WK1;	ADI02905, YP_003703470;		
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	YP_003874669, ADN02396;		
	<i>Caldicellulosiruptor saccharolyticus</i> DSM 8903; <i>E. coli</i> ;	YP_002315593;		
	<i>Deferribacter desulfuricans</i> SSM1;	YP_002572562;		
	<i>Thermosynechococcus elongatus</i> BP-1;	YP_001179645, ABP66454;		
	<i>Hydrogenobacter thermophilus</i> TK-6;	ADR29155, YP_001460564;		
	<i>Nostoc punctiforme</i> PCC 73102;	YP_003496880, BAI81124;		
	' <i>Nostoc azollae</i> ' 0708;	NP_681848, BAC08610;		
	<i>Arthrospira maxima</i> CS-328;	YP_003431766, BAI68565;		
	<i>Prochlorococcus marinus</i> str. MIT 9301; <i>Cyanobium</i> sp. PCC 7001;	ACC82168, ADN14191;		
	<i>Synechococcus</i> sp. PCC 7335;	ADI62939;		
	<i>Arthrospira platensis</i> str. Paraca;	EDZ97146;		
	<i>Microcystis aeruginosa</i> NIES-843;	AB017457;		
	<i>Chlamydomonas reinhardtii</i> ;	ZP_05044537, EDY37846;		
	<i>Arabidopsis thaliana</i> ;	ZP_05037932;		
	<i>Oryza sativa</i> Indica Group;	ZP_06383646;		
	<i>Glycine max</i> ;	BAG02689;		
	<i>Schizosaccharomyces japonicus</i> yFS275;	XP_001693179, EDP03205;		
	56: 2-Methylbutyraldehyde reductase (EC 1.1.1.265)	<i>Pichia pastoris</i> GS115;	XP_002490018, CAY67737,	
		<i>Saccharomyces cerevisiae</i> S288c;	XM_002489973;	
		<i>Aspergillus fumigatus</i> Af293;	DAA12209, NP_010656 ,	
		<i>Debaryomyces hansenii</i> CBS767;	NM_001180676 ;	
		<i>Debaryomyces hansenii</i>	XP_752003;	
		<i>Kluyveromyces lactis</i> ;	XP_002770138;	
		<i>Lachancea thermotolerans</i> CBS 6340;	CAR65507;	
		<i>Lachancea thermotolerans</i> ;	CAH02579;	
		<i>Saccharomyces cerevisiae</i> EC1118;	XP_002554884;	
		<i>Saccharomyces cerevisiae</i> JAY291;	CAR24447, CAR23718;	
		<i>Saccharomyces cerevisiae</i> S288c;	CAY78868;	
		57: 3-Methylbutanal reductase (EC 1.1.1.265)	<i>Saccharomyces cerevisiae</i> EC1118;	EEU08013;
			<i>Saccharomyces cerevisiae</i> JAY291;	DAA10635, NM_001183405,
			<i>Geobacillus kaustophilus</i> HTA426;	NP_014490;
			<i>Azohydromonas lata</i> ;	CAY86141;
<i>Rhodospirillum rubrum</i> ATCC 29411;	EEU07090;			
07: 3-Ketothiolase (reversible)	<i>Rhodospirillum rubrum</i> ATCC 29411;	YP_147173, BAD75605;		
	<i>Allochrochromatium vinosum</i> ;	YP_523526;		
	<i>Dechloromonas aromatica</i> RCB;	CAA01849, CAA01846;		
	<i>Rhodobacter sphaeroides</i> ATCC 17029;	YP_286222;		
	<i>Rhodobacter sphaeroides</i> ATCC 17025;	YP_001041914;		
	<i>Bacillus</i> sp. 256;	YP_001166229;		
	<i>Silicibacter lacuscaerulensis</i> ITI-1157;	ABX11181;		
		ZP_05785678;		

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
	<i>Aspergillus fumigatus</i> Af293;	XP_752635;
	<i>Rhizobium etli</i> ;	AAK21958;
	<i>Citricella</i> sp. SE45;	ZP_05784120, ZP_05781517;
	<i>Silicibacter</i> sp. TrichCH4B;	ZP_05742998;
	<i>Azohydromonas lata</i> ;	AAC83659, AAD10275;
	<i>Chromobacterium violaceum</i> ;	AAC69616;
	<i>Dinoroseobacter shibae</i> DFL 12;	ABV95064;
	<i>Alcaligenes</i> sp. SH-69;	AAP41838;
	<i>Candida dubliniensis</i> CD36;	CAX43351, XP_002418052;
	<i>Pseudomonas</i> sp. 14-3;	CAK18903;
	<i>Aspergillus flavus</i> NRRL3357;	XP_002375989;
	<i>Aedes aegypti</i> ;	EAT37298, EAT37297,
		XP_001654752, XP_001654751;
	<i>Scheffersomyces stipitis</i> CBS 6054;	ABN68380, XP_001386409;
	<i>Cyanothece</i> sp. PCC 7424;	YP_002375827, ACK68959;
	<i>Cyanothece</i> sp. PCC 7822;	YP_003886602, ADN13327;
	<i>Microcystis aeruginosa</i> NIES-843;	BAG04828;
08':	<i>Syntrophothermus lipocalidus</i> DSM	YP_003702743, ADI02178,
3-Hydroxyacyl-CoA	12680;	ADI01287, ADI01071;
dehydrogenase	<i>Oceanithermus profundus</i> DSM 14977;	ADR36325;
	<i>Anoxybacillus flavithermus</i> WK1;	YP_002317076, YP_002315864;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001210823, BAF58454;
	<i>Geobacillus kaustophilus</i> HTA426;	YP_149248, YP_147889;
	<i>Deferribacter desulfuricans</i> SSM1;	YP_003497047, BAI81291;
	<i>Glomerella graminicola</i> M1.001;	EFQ32520, EFQ35765;
	<i>Legionella pneumophila</i> str. Corby;	YP_001250712, ABQ55366;
	<i>Aspergillus fumigatus</i> Af293;	XP_748706, XP_748351;
	<i>Coprinopsis cinerea</i> okayama7#130;	EAU80763;
	<i>Botryotinia fuckeliana</i> B05.10;	XP_001559519;
	<i>Coccidioides posadasii</i> ; <i>E. coli</i> ;	ABH10642; YP_001462756;
	<i>Chelativorans</i> sp. BNC1;	YP_675197;
	<i>Nostoc punctiforme</i> PCC 73102;	ACC81853, YP_001866796;
	<i>Oscillatoria</i> sp. PCC 6506;	ZP_07114022, CBN59220;
09':	<i>Bordetella petrii</i> ;	CAP41574;
Enoyl-CoA dehydratase	<i>Bordetella petrii</i> DSM 12804;	YP_001629844;
	<i>Anoxybacillus flavithermus</i> WK1;	YP_002315700, YP_002314932;
	<i>Geobacillus kaustophilus</i> HTA426;	YP_148541, YP_147845,
	<i>Geobacillus kaustophilus</i> ;	BAD76199; BAD18341;
	<i>Syntrophothermus lipocalidus</i> DSM	ADI02939, ADI02740,
	12680;	ADI02007, ADI01364;
	<i>Acinetobacter</i> sp. SE19;	AAG10018;
	<i>Scheffersomyces stipitis</i> CBS 6054;	ABN64617, XP_001382646;
	<i>Laccaria bicolor</i> S238N-H82;	EDR09131, XP_001888157;
	<i>Alternaria alternata</i> ;	BAH83503,
	<i>Ajellomyces dermatitidis</i> ER-3;	EEQ91989;
	<i>Aspergillus fumigatus</i> Af293;	EAL93360, XP_755398;
	<i>Cryptococcus neoformans</i> var.	XP_572730;
	<i>neoformans</i> JEC21; <i>E. Coli</i> ;	ADN73405, YP_001458194;
	<i>Aspergillus flavus</i> NRRL3357;	XP_002377859;
	<i>Laccaria bicolor</i> S238N-H82;	EDR01115;
	<i>Neosartorya fischeri</i> NRRL 181;	EAW18645;
	<i>Nostoc</i> sp. 'Peltigera membranacea	ADA69246;
	<i>cyanobiont</i> ';	
10':	<i>Xanthomonas campestris</i> pv.	CAP53709;
2-Enoyl-CoA reductase	<i>Campestris</i> ; <i>Xanthomonas campestris</i>	YP_001905744;
	pv. <i>campestris</i> str. B100; <i>Xanthomonas</i>	ZP_06489037;
	<i>campestris</i> pv. <i>musacearum</i>	
	NCPPB4381; <i>Xanthomonas campestris</i>	ZP_06487845;
	pv. <i>vasculorum</i> NCPPB702;	
	<i>Aeromicrobium marinum</i> DSM 15272;	ZP_07718056, EFQ82338;
	<i>Rhodobacteriales bacterium</i> HTCC2083;	ZP_05074461, EDZ42121;
	<i>Lysinibacillus fusiformis</i> ZC1;	
	<i>Mycobacterium smegmatis</i> str. MC2	ZP_07049092, EFI69525;
	155;	YP_886510, ABK76225;
	<i>Lysinibacillus sphaericus</i> C3-41;	
	<i>Coprinopsis cinerea</i> okayama7#130;	YP_001699417, ACA41287;
	<i>Arthroderma gypseum</i> CBS 118893;	XP_002910885, EFI27391;
	<i>Paracoccidioides brasiliensis</i> Pb01;	EFR05506;
	<i>Paracoccidioides brasiliensis</i> Pb18;	XP_002796528, EEH39074;
	<i>Ajellomyces capsulatus</i> G186AR;	EEH43955;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.			
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation	
11': Acyl-CoA reductase (EC 1.2.1.50)	<i>Ostreococcus tauri</i> ; <i>Jatropha curcas</i> ;	EEH03439; XP_003083795, CAL57762; ACS32302;	
	<i>Clostridium cellulovorans</i> 743B; <i>Thermosphaera aggregans</i> DSM 11486; <i>Delftia acidovorans</i> SPH-1;	YP_003845606, ADL53842; YP_003649571, ADG90619;	
	<i>Comamonas testosteroni</i> KF-1; <i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697;	YP_001565543, ABX37158; YP_03543536; YP_002321654, ACJ51276;	
	<i>Clostridium papyrosolvans</i> DSM 2782; <i>Acidovorax avenae</i> subsp. <i>avenae</i> ATCC 19860;	ZP_05497968, EEU57047; ZP_06211782, EFA39209;	
	<i>Comamonas testosteroni</i> KF-1; <i>Aminomonas paucivorans</i> DSM 12260; <i>Herpetosiphon aurantiacus</i> ATCC 23779;	EED67822; ZP_07740542, EFQ24431 ; ABX07240, YP_001547368;	
	<i>Clostridium beijerinckii</i> NCIMB 8052; <i>Geobacillus</i> sp. G11MC16;	ABR34265, YP_001309221; ZP_03148237, EDY05596;	
	<i>Clostridium lentocellum</i> DSM 5427; <i>Leadbetterella byssophila</i> DSM 17132; <i>Actinosynnema mirum</i> DSM 43827;	ZP_06885967, EFG96716; YP_003997212, ADQ16859;	
	<i>Haliangium ochraceum</i> DSM 14365; <i>Photobacterium phosphoreum</i> ;	YP_003101455, ACU37609 ; ACY16972, YP_003268865;	
	<i>Simmondsia chinensis</i> ; <i>Hevea brasiliensis</i> ;	AAT00788; AAD38039;	
	<i>Arabidopsis thaliana</i> ;	AAR88762; ABE65991; ACZ56328;	
	12': Hexanol dehydrogenase	<i>Mycobacterium chubuense</i> NBB4;	ACZ56328;
	12'': Octanol dehydrogenase EC 1.1.1.73	<i>Drosophila subobscura</i> ;	ABO61862, ABO65263, CAD43362, CAD43361, CAD54410, CAD43360, CAD43359, CAD43358 CAD43357, CAD43356;
	43': Short chain alcohol dehydrogenase	<i>Pyrococcus furiosus</i> DSM 3638; <i>Burkholderia vietnamiensis</i> G4; <i>Geobacillus thermoleovorans</i> ; <i>Geobacillus kaustophilus</i> HTA426; <i>Anoxybacillus flavithermus</i> WK1; <i>Helicobacter pylori</i> PeCan4; <i>Mycobacterium chubuense</i> NBB4; <i>Mycobacterium avium</i> subsp. <i>avium</i> ATCC 25291; <i>Aspergillus oryzae</i> ; <i>cyanobacterium</i> UCYN-A; <i>Anabaena circinalis</i> AWQC131C; <i>Cylindrospermopsis raciborskii</i> T3; <i>Helicobacter pylori</i> Sat464; <i>Helicobacter pylori</i> Cuz20; <i>Mycobacterium intracellulare</i> ATCC 13950; <i>Mycobacterium avium</i> subsp. <i>avium</i> ATCC 25291; <i>Gluconacetobacter hansenii</i> ATCC 23769; <i>Helicobacter pylori</i> Shi470; <i>Mycobacterium avium</i> 104; <i>Citrus sinensis</i> ; <i>Gossypium hirsutum</i> ; <i>Arabidopsis halleri</i> ; <i>Paracoccidioides brasiliensis</i> Pb01; <i>Pyrenophora tritici-repentis</i> Pt-1C-BFP; <i>Ajellomyces capsulatus</i> H143; <i>Scheffersomyces stipitis</i> CBS 6054; <i>Ralstonia eutropha</i> H16;	AAC25556; ABO56626; BAA94092; YP_146837, BAD75269; YP_002314715, ACJ32730; YP_003927327, ADO07277; ACZ56328; ZP_05215778; BAE71320; YP_003421738, ADB95357; ABI75134; ABI75108; ADO05766; ADO04259; ZP_05228059, ZP_05228058; ZP_05215779; ZP_06834730, EFG83978; YP_001910563, ACD48533; YP_880627, ABK67217; ADH82118; ABD65462; ABZ02361, ABZ02360; XP_002792148, EEH34889; XP_001940779, EDU43498; EER38733; XP_001382930, ABN64901; NP_942643 (hoxK), NP_942644 (hoxG), YP_015633 (hoxZ); AAP85757 (hoxK), AAP85758 (hoxG), AAA16463 (hoxZ); ABF08183 (hoxK), YP_583451 (hoxG), ABF08182 (hoxG); ADK12981, ADK12980; ACJ15972; YP_004763067;
	70: Membrane-bound hydrogenase (MBH)	<i>Ralstonia eutropha</i> H16; <i>Cupriavidus metallidurans</i> CH34; <i>Thiocapsa roseopersicina</i> , <i>Thermococcus onnurineus</i> NA1;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
71: Soluble hydrogenase (SH) (NAD(P)-reducing)	<i>Thermococcus</i> sp. 4557;	YP_004763083;
	<i>Thermococcus</i> sp. 4557;	YP_004763081;
	<i>Thermococcus</i> sp. 4557;	AEK73406;
	<i>Thermococcus</i> sp. 4557;	AEK73404;
	<i>Thermococcus</i> sp. 4557;	NP_579163;
	<i>Pyrococcus furiosus</i> DSM 3638;	NP_579162;
	<i>Pyrococcus furiosus</i> DSM 3638;	YP_004624085;
	<i>Pyrococcus yayanosii</i> CH1;	YP_004624086;
	<i>Pyrococcus yayanosii</i> CH1;	YP_004624087;
	<i>Pyrococcus yayanosii</i> CH1;	NP_142896;
	<i>Pyrococcus horikoshii</i> OT3;	BAK19334;
	<i>Hydrogenovibrio marinus</i> ;	CAA63615;
	<i>Alcaligenes</i> sp.;	CAA63616;
	<i>Rubrivivax</i> sp.;	BAF73677;
	<i>Hydrogenobacter thermophilus</i> TK-6;	ACS32538;
	<i>Thermococcus gammatolerans</i> EJ3;	ADN36337;
	<i>Methanoplanus petrolearius</i> DSM 11571;	YP_002958402;
	<i>Thermococcus gammatolerans</i> EJ3;	YP_004638463 (hoxZ);
	<i>Oligotropha carboxidovorans</i> OM5;	AEI08136 (hoxZ);
	<i>Aquifex aeolicus</i> VF5;	NP_213456 (hoxZ);
	<i>Centipeda periodontii</i> DSM 2778;	ZP_08500995 (hoxZ);
	<i>Selenomonas noxia</i> ATCC 43541;	ZP_06602778 (hoxZ);
	<i>Allochromatium vinosum</i> DSM 180;	ADC63224 (hoxZ);
	<i>Thiomonas intermedia</i> K12;	ADG32404 (hoxZ);
	<i>Aquifex aeolicus</i> VF5;	AAC06857 (hoxZ);
	<i>Ralstonia eutropha</i> H16;	AAP85843 (hoxY), AAP85844 (HoxH);
	<i>Ralstonia eutropha</i> H16;	NP_942730 (hoxH), NP_942729 (hoxY);
	<i>Ralstonia eutropha</i> H16;	NP_942727 (hoxF), NP_942728 (hoxU);
	<i>Ralstonia eutropha</i> H16;	AAP85841 (hoxF), AAP85842 (hoxU);
	<i>Ralstonia eutropha</i> H16;	AAC06140 (hoxF), AAC06141 (hoxU), AAC06142 (hoxY), AAC06143 (hoxH);
	<i>Ralstonia eutropha</i> H16;	AAD38065 (hoxH);
	<i>Rhodobacter capsulatus</i> ;	YP_002797671 (hoxH);
	<i>Azotobacter vinelandii</i> DJ;	BAG01243 (hoxH);
	<i>Microcystis aeruginosa</i> NIES-843;	ABW32682 (hoxH);
	<i>Acaryochloris marina</i> MBIC11017;	AAN03569 (hoxH);
	<i>Synechococcus</i> sp. PCC 7002;	CAA66383 (hoxH);
	<i>Synechococcus elongatus</i> PCC 6301;	CAA66382 (hoxY);
	<i>Synechococcus elongatus</i> PCC 6301;	AAX89151 (hoxY);
	<i>Allochromatium vinosum</i> ;	CAO88137 (hoxY);
	<i>Microcystis aeruginosa</i> PCC 7806;	YP_002797670 (hoxY);
	<i>Azotobacter vinelandii</i> DJ;	CAA66381 (hoxU);
	<i>Synechococcus elongatus</i> PCC 6301;	AAX89150 (hoxU);
	<i>Allochromatium vinosum</i> ;	ABC26909 (hoxU);
	<i>Arthrospira platensis</i> FACHB341;	CAO88140 (hoxU);
	<i>Microcystis aeruginosa</i> PCC 7806;	AAY57574 (hoxU);
	<i>Lyngbya majuscula</i> CCAP 1446/4;	YP_172263 (hoxU);
	<i>Synechococcus elongatus</i> PCC 6301;	YP_001803733 (hoxU);
	<i>Cyanothece</i> sp. ATCC 51142;	CAA73873 (hoxF);
	<i>Synechococcus elongatus</i> PCC 6301;	AAX89149 (hoxF);
	<i>Allochromatium vinosum</i> ;	ABC26907 (hoxF);
	<i>Arthrospira platensis</i> FACHB341;	YP_001733465 (hoxF);
	<i>Synechococcus</i> sp. PCC 7002;	BAJ63286 (hoxH);
	<i>Anaerolinea thermophila</i> UNI-1;	CCC57856 (hoxF);
	<i>Caloramator australicus</i> RC3;	NP_942649 (hoxO), AAP85763 (hoxO), AAA16467 (hoxO);
	<i>Ralstonia eutropha</i> H16;	ABF08176 (hoxO), YP_583445 (hoxO);
	<i>Ralstonia eutropha</i> H16;	NP_942650 (hoxQ), AAP85764 (hoxQ), AAA16468 (hoxQ);
	<i>Ralstonia eutropha</i> H16;	ABF08175 (hoxQ), YP_583444 (hoxQ);
	<i>Cupriavidus metallidurans</i> CH34;	AAA19504 (hoxQ);
	<i>Azotobacter vinelandii</i> ;	EHC91928 (hoxQ/hoxR), EFX49216 (hoxQ/hoxR), ZP_06652932 (hoxQ);
	<i>Salmonella enterica</i> subsp.;	ZP_08506135 (hoxQ);
	<i>Escherichia coli</i> B354;	
	<i>Methyloversatilis universalis</i> FAM5;	

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
	<i>Shigella flexneri</i> CDC 796-83; <i>Ralstonia eutropha</i> H16;	EFW61888 (hoxQ); AAA16469 (hoxR); NP_942651 (hoxR);
	<i>Azotobacter vinelandii</i> ; <i>Ralstonia eutropha</i> H16;	AAA19505 (hoxR); NP_942652 (hoxT), AAP85766 (hoxT), AAA16470 (hoxT);
	<i>Cupriavidus metallidurans</i> CH34; <i>Azotobacter vinelandii</i> DJ;	ABF08173 (hoxT); YP_002802114 (hoxT), ACO1139 (hoxT);
	<i>Ralstonia eutropha</i> H16;	NP_942648 (hoxL), AAP85762 (hoxL), AAA16466 (hoxL);
	<i>Azotobacter vinelandii</i> ; <i>Oligotropha carboxidovorans</i> OM5; <i>Cupriavidus metallidurans</i> CH34; <i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Weltevreden str. 2007-60-3289- 1; <i>Oligotropha carboxidovorans</i> OM5; <i>Oligotropha carboxidovorans</i> OM4; <i>Azotobacter vinelandii</i> DJ;	AAA19502 (hoxL); YP_015634 (hoxL); ABF08177 (hoxL), YP_583446 (hoxL); CBY95754 (hoxL); YP_004638464 (hoxL); AEI04509 (hoxL); YP_002802118 (hoxL), ACO81143 (hoxL); ZP_08506137 (hoxL), EGK70316 (hoxL);
	<i>Methyloversatilis universalis</i> FAM5;	NP_942653 (hoxV), AAP85767 (hoxV), AAA16471 (hoxV);
	<i>Ralstonia eutropha</i> H16;	AAA19507 (hoxV);
	<i>Azotobacter vinelandii</i> ; <i>Oligotropha carboxidovorans</i> OM5; <i>Cupriavidus metallidurans</i> CH34; <i>Azotobacter vinelandii</i> DJ;	YP_015636 (hoxV); ABF08172 (hoxV); YP_002802113 (hoxV);
	<i>Cupriavidus metallidurans</i> CH34; <i>Methyloversatilis universalis</i> FAM5; <i>Methyloversatilis universalis</i> FAM5;	YP_583441 (hoxV); ZP_08506132 (hoxV); EGK70311 (hoxV);
	<i>Ralstonia eutropha</i> H16 <i>Oligotropha carboxidovorans</i> OM5, <i>Oligotropha carboxidovorans</i> OM4; <i>Azotobacter vinelandii</i> ; <i>Azotobacter vinelandii</i> DJ;	NP_942647 (hoxM); YP_004638462 (hoxM); AEI04507 (hoxM); AAA19501 (hoxM); YP_002802119 (hoxM);
	<i>Cupriavidus metallidurans</i> CH34; <i>Hydrogenobacter thermophilus</i> TK-6; <i>Hydrogenobacter thermophilus</i> TK-6; <i>Thermoproteus tenax</i> Kra 1; <i>Acidithiobacillus</i> sp. GGI-221;	YP_583447 (hoxM); BAF73673 (hoxM); YP_003432119 (hoxM); CCC80713 (hoxM); EGQ60729 (hoxM);
	<i>Methyloversatilis universalis</i> FAM5; <i>Burkholderiales bacterium</i> 1_1_47; <i>Thiomonas intermedia</i> K12; <i>Thermococcus gammatolerans</i> EJ3;	ZP_07342912 (hoxM); YP_003644737 (hoxM); YP_002958602 (hybD/hycI/hoxM);
	<i>Ralstonia eutropha</i> H16; <i>Azorhizobium caulinodans</i> ORS 571; <i>Bradyrhizobium japonicum</i> ; <i>Hyphomicrobium</i> sp. MC1;	NP_942661 (hoxA), AAP85775; AAS91037 (hoxA); CAA78991 (hoxA); YP_004674255 (hoxA);
	<i>Azoarcus</i> sp. BH72; <i>Methyloversatilis universalis</i> FAM5; <i>Grimontia hollisae</i> CIP 101886; <i>Oxalobacteraceae bacterium</i> ;	YP_935307 (hoxA); ZP_08506123 (hoxA); ZP_06053565 (hoxA); ZP_08276168 (hoxA);
	<i>Ralstonia eutropha</i> H16; <i>Azoarcus</i> sp. BH72; <i>Oligotropha carboxidovorans</i> OM5; <i>Ralstonia eutropha</i> H16;	NP_942662 (hoxB), AAP85776; YP_935309 (hoxB); YP_004638467 (hoxB); AAP85777 (hoxC), NP_942663;
	<i>Azoarcus</i> sp. BH72; <i>Oligotropha carboxidovorans</i> OM4; <i>Oligotropha carboxidovorans</i> OM5; <i>Oxalobacteraceae bacterium</i> IMCC9480;	YP_935310 (hoxC); AEI04502 (hoxC); YP_004638457 (hoxC); ZP_08276171 (hoxJ), EGF30361 (hoxJ);
	<i>Alcaligenes hydrogenophilus</i> ; <i>Synechocystis</i> sp. PCC 6803;	AAB49362 (hoxJ); BAA18357 (hypA);
	<i>Ralstonia eutropha</i> H16; <i>Ralstonia eutropha</i> H16; <i>Ralstonia eutropha</i> H16;	NP_942654 (hypA1); NP_942733 (hypA2); NP_942716 (hypA3);
	<i>Cupriavidus metallidurans</i> CH34; <i>Ralstonia eutropha</i> H16;	YP_583440 (hypA); NP_942655 (hypB1);

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
	<i>Ralstonia eutropha</i> H16;	AAP85769 (hypB1);
	<i>Butyrivibrio proteoclasticus</i> B316;	YP_003830670 (hypB1);
	<i>Oligotropha carboxidovorans</i> OM5;	YP_004638455 (hypB);
	<i>Oligotropha carboxidovorans</i> OM4;	AEI04500 (hypB);
	<i>Desulfotobacterium metallireducens</i> DSM 15288;	ZP_08976390 (hypB),
	<i>Synechocystis</i> sp. PCC 6803;	EHC20145 (hypB);
	<i>Cyanothece</i> sp. CCY0110;	BAA18180 (hypC);
	<i>Cupriavidus metallidurans</i> CH34,	EAZ91066 (hypC);
	<i>Ralstonia eutropha</i> H16;	ABF08421(hypC);
	<i>Ralstonia eutropha</i> H16;	NP_942657 (hypC1);
	<i>Ralstonia eutropha</i> H16;	AAP85826 (hypC2);
	<i>Ralstonia eutropha</i> H16;	CAA49734 (hypD);
	<i>Cupriavidus metallidurans</i> CH34;	YP_583436 (hypD);
	<i>Cupriavidus metallidurans</i> CH34;	ABF08422 (hypD);
	<i>Escherichia coli</i> BL21(DE3);	ACT44398 (hypD);
	<i>Synechocystis</i> sp. PCC 6803;	BAA17478 (hypE);
	<i>Ralstonia eutropha</i> H16;	CAA49735 (hypE);
	<i>Ralstonia eutropha</i> H16;	NP_942659 (hypE1);
	<i>Ralstonia eutropha</i> H16;	AAP85829 (hypE2);
	<i>Rhizobium leguminosarum</i> ;	CAA37164 (hypE);
	<i>Azotobacter vinelandii</i> ;	AAA19513 (hypE);
	<i>Aeropyrum permix</i> K1;	NP_148343 (hypE);
	<i>Sulfolobus solfataricus</i> P2;	NP_341628 (hypE);
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003432665 (hypE);
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001212249 (hypE);
	<i>Syntrophothermus lipocalidus</i> DSM 12680;	ADI01176 (hypE),
	<i>Hydrogenobacter thermophilus</i> TK-6;	YP_003701741 (hypE);
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_003432667 (hypF);
	<i>Syntrophothermus lipocalidus</i> DSM 12680;	YP_001212246 (hypF);
	<i>Caldicellulosiruptor bescii</i> DSM 6725;	ADI01173 (hypF),
	<i>Ralstonia eutropha</i> H16;	YP_003701738 (hypF);
	<i>Ralstonia eutropha</i> H16;	YP_002572964 (hypF);
	<i>Ralstonia eutropha</i> H16;	CAA49731 (hypF);
	<i>Hydrogenobacter thermophilus</i> TK-6;	NP_942660 (hypX);
	<i>Rhizobium leguminosarum</i> ;	AAP85774 (hypX)
	<i>Methyloversatilis universalis</i> FAM5;	YP_003433460 (hypX);
	<i>Cupriavidus metallidurans</i> CH34;	CAA37165 (hypX);
	<i>Ralstonia eutropha</i> H16;	ZP_08506124 (hoxX);
73:	<i>Desulfobulbus propionicus</i> DSM 2032;	ABF08424 (hoxX);
NAD(P)-dependent	<i>Acetohalobium arabaticum</i> DSM 5501;	CAA52735 (hoxX);
hydrogenase	<i>Ilyobacter polyt</i> ; ropus DSM 2926; beta proteobacterium KB13	ADY56959, YP_004195043;
	<i>Acetohalobium arabaticum</i> DSM 5501;	YP_003826884;
	<i>Moorella thermoacetica</i> ATCC 39073;	ADO82414;
	<i>Moorella thermoacetica</i> ATCC 39073;	EDZ65062, ZP_05082375;
	<i>Moorella thermoacetica</i> ;	ADL11819
74:	<i>Methanosaeta harundinacea</i> 6Ac;	YP_429324, ABC18781;
Formate dehydrogenase	<i>Methanoculleus marisnigri</i> JR1;	YP_431142, ABC20599;
using NAD(P)H	<i>Methanocorpusculum labreanum</i> Z;	AAB18330 (α), AAB18329 (β);
	<i>Helicobacter bilis</i> ATCC 43879;	AET63712, AET63711,
	<i>Helicobacter bilis</i> ATCC 43879;	YP_001047290;
	<i>Pelotomaculum thermopropionicum</i> SI;	YP_001029904, YP_001029903;
	<i>Hydrogenobacter thermophilus</i> TK-6;	ZP_04582064 (NADPH);
	<i>Hydrogenobacter thermophilus</i> TK-6;	EEO23341 (NADPH);
	<i>Klebsiella variicola</i> At-22;	YP_001213196;
	<i>Azospirillum</i> sp. B510;	YP_003432807;
	<i>Thermococcus gammatolerans</i> EJ3;	YP_003433330 (NDA dependent);
	<i>Yersinia pestis</i> Antiqua;	ADC58081, YP_003439113;
	<i>Thermofilum pendens</i> Hrk 5;	YP_003451652, YP_003450092;
	<i>Ferrimonas balearica</i> DSM 9799;	YP_002958615;
	<i>Thermodesulfatator indicus</i> DSM 15286;	ABG15899;
	<i>Shewanella baltica</i> BA175;	YP_919603;
	<i>Methanocella paludicola</i> SANAE;	YP_003913071;
	<i>Methanosaeta harundinacea</i> 6Ac;	AEH46025;
		AEG12633;
		YP_003357462, YP_003357461;
		AET64643, AET64987,
		AET65705;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
75: 10-Formyl-H ₄ folate synthetase (ADP forming, 10-Formyltetrahydrofolate Synthetase)	<i>Moorella thermoacetica</i> ATCC 39073; <i>Methanocorpusculum labreanum</i> Z; <i>Sphingomonas paucimobilis</i> ; <i>Desulfatibacillum alkenivorans</i> AK-01; <i>Corynebacterium aurimucosum</i> ; <i>Clostridium acidurici</i> ; <i>Sphingobium</i> sp. SYK-6; <i>Listeria monocytogenes</i> serotype 4b str. CLIP 80459; <i>Vibrio fischeri</i> MJ11; <i>Anoxybacillus flavithermus</i> WK1; <i>Thermotoga lettingae</i> TMO; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Thermosiphon melanesiensis</i> BI429; <i>Thermotoga petrophila</i> RKU-1 <i>Pelotomaculum thermopropionicum</i> SI;	YP_428991; YP_001030445; BAD61061; ACL05327; YP_002834788; AAA53187; YP_004834408; YP_002758587; YP_002156619; YP_002315932; YP_001471133; YP_001410584; YP_001305561; YP_001244647 YP_001210750; YP_430368, ABC19825;
76: 5,10-Methenyl-H ₄ folate cyclohydrolase (Methenyltetrahydrofolate cyclohydrolase)	<i>Moorella thermoacetica</i> ATCC 39073; <i>Thermotoga lettingae</i> TMO; <i>Caldicellulosiruptor bescii</i> DSM 6725; <i>Thermotoga petrophila</i> RKU-1; <i>Anoxybacillus flavithermus</i> WK1; <i>Geobacillus kaustophilus</i> HTA426; <i>Geobacillus kaustophilus</i> HTA426; <i>Synechococcus</i> sp. JA-2-3B'a(2-13); <i>Synechococcus</i> sp. JA-3-3Ab; <i>Exiguobacterium</i> sp. AT1b; <i>Thermotoga lettingae</i> TMO;	ABV34070; YP_002572856; ABQ47072; YP_002315305; BAD76681; YP_148249; YP_476354; YP_475381; YP_002884899; YP_001471134; ABC19825, YP_430368; BAD76681;
77: 5,10-Methylene-H ₄ folate dehydrogenase	<i>Moorella thermoacetica</i> ATCC 39073; <i>Geobacillus kaustophilus</i> HTA426; <i>Syntrophothermus lipocalidus</i> ; <i>Caldicellulosiruptor kronotskyensis</i> ; <i>Caldicellulosiruptor kristjanssonii</i> ; <i>Caldicellulosiruptor hydrothermalis</i> ; <i>Caldicellulosiruptor owensensis</i> OL; <i>Caldicellulosiruptor hydrothermalis</i> ; <i>Kosmotoga olearia</i> TBF 19.5.1; <i>Exiguobacterium</i> sp. AT1b; <i>Komagataella pastoris</i> CBS 7435; <i>Homo sapiens</i> ; <i>Taeniopygia guttata</i> ; <i>Syntrophobotulus glycolicus</i> DSM 8271; <i>Olsenella uli</i> DSM 7084;	ADI01214; ADQ46551; ADQ40482; ADQ07463; ADQ04336; YP_003992832; ACR80790; ACQ69454; CCA37557; AAH09806; XP_002200380; ADY56189; ADK67906; YP_430048, ABC19505;
78: 5,10-Methylene-H ₄ folate reductase (Methylenetetrahydrofolate reductase)	<i>Moorella thermoacetica</i> ATCC 39073; <i>Syntrophothermus lipocalidus</i> ; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Thermotoga petrophila</i> RKU-1; <i>Fervidobacterium nodosum</i> Rt17-B1; <i>Thermotoga lettingae</i> TMO; <i>Thermosiphon melanesiensis</i> BI429; <i>Synechococcus</i> sp. JA-2-3B'a(2-13); <i>Hippea maritima</i> DSM 10411; <i>Spirochaeta thermophila</i> DSM 6192; <i>Deferribacter desulfuricans</i> SSM1; <i>Hydrogenobacter thermophilus</i> TK-6; <i>Pelotomaculum thermopropionicum</i> SI;	ADQ04336; YP_003992832; ACR80790; ACQ69454; CCA37557; AAH09806; XP_002200380; ADY56189; ADK67906; YP_430048, ABC19505; ADI02156; ABS61421; ABQ46674; ABS61126; ABV33918; YP_001305980; YP_477166; YP_004340445; YP_003875363; YP_003496368; YP_003432279; BAF59187, YP_001211556;
79: Methyl-H ₄ folate: corrinoid iron-sulfur protein Methyltransferase (Methyltetrahydrofolate:corrinoid iron-sulfur protein Methyltransferase)	<i>Moorella thermoacetica</i> ATCC 39073; <i>Pelotomaculum thermopropionicum</i> SI; <i>Clostridium carboxidivorans</i> P7; <i>Desulfotobacterium hafniense</i> DCB-2; <i>Dinoroseobacter shibae</i> DFL 12; <i>Ammonifex degensii</i> KC4; <i>Desulfotomaculum acetoxidans</i> ; <i>Rhodobacter sphaeroides</i> KD131; <i>Carboxydotherrmus hydrogenoformans</i> ; <i>Rhodobacter sphaeroides</i> 2.4.1; <i>Heliobacterium modesticaldum</i> Ice1; <i>Sinorhizobium meliloti</i> 1021; <i>Acetonema longum</i> DSM 6540	YP_430950, YP_430174; YP_001211554; ADO12092; YP_002461301; YP_001533020; YP_003238352; YP_003190781; YP_002525435; YP_360065; YP_352826; YP_001680302; NP_386092; ZP_08625620;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
80: Corrinoid iron-sulfur protein (CFeSP)	<i>Moorella thermoacetica</i> ; <i>Carboxydotherrmus hydrogenoformans</i> <i>Clostridium ragsdalei</i> ; <i>Clostridium autoethanogenum</i> ; <i>Clostridium sticklandii</i> DSM 519; <i>Clostridium sticklandii</i> ;	AAA23255; 2H9A_A, 2H9A_B; AEI90763, AEI90762; AEI90746, AEI90745; YP_003936194; CBH21289;
81: CO dehydrogenase/acetyl-CoA synthase (Fd ²⁻)	<i>Moorella thermoacetica</i> ATCC 39073; <i>Moorella thermoacetica</i> ATCC 39073; <i>Moorella thermoacetica</i> ; <i>Caldicellulosiruptor kristjanssonii</i> ; <i>Caldicellulosiruptor saccharolyticus</i> ; <i>Clostridium ragsdalei</i> ; <i>Clostridium autoethanogenum</i> ; <i>Desulfosporosinus orientis</i> DSM 765; <i>Methanococcus aeolicus</i> Nankai-3; <i>Desulfobacca acetoxidans</i> DSM 11109; <i>Thermodesulfatator indicus</i> ; <i>Acetohalobium arabaticum</i> DSM 5501; <i>Desulfarculus baarsii</i> DSM 2075; <i>Archaeoglobus veneficus</i> SNP6; <i>Methanosalsum zhilinae</i> DSM 4017; <i>Thermosediminibacter oceani</i> ; <i>Desulfotomaculum kuznetsovii</i> ; <i>Methanosalsum zhilinae</i> DSM 4017; <i>Thermodesulfobium narugense</i> ;	ABC19516, YP_430059; YP_430813 (CODH); AAA23229, AAA23228; ADQ39747; YP_001179230; AEI90761; AEI90744; AET68776; ABR56750; YP_004370981; AEH46031; ADL12817; YP_003806211; YP_004341848; AEH60991; ADL07576; YP_004517493, YP_004516875; AEH60989, AEH60993; YP_004437266;
82: Pyruvate synthase (Fd ²⁻)	<i>Desulfobacca acetoxidans</i> ; <i>Archaeoglobus veneficus</i> SNP6; <i>Hippea maritima</i> DSM 10411; <i>Desulfurobacterium thermolithotrophum</i> ; <i>Archaeoglobus veneficus</i> ; <i>Thermodesulfobium narugense</i> ; <i>Archaeoglobus veneficus</i> SNP6; <i>Thermobacillus composti</i> KWC4; <i>Desulfobacca acetoxidans</i> ; <i>Methanolinea tarda</i> NOBI-1; <i>Methanobacterium</i> sp. AL-21; <i>Methanocella paludicola</i> SANAE; <i>Methanothermobacter marburgensis</i> str. Marburg;	YP_004370392; YP_004341929; YP_004339618; YP_004281767, YP_004281766, ADY73708; AEA47214; AEE14134; YP_004341930; ZP_08918406; AEB09210; EHF09898; YP_004289712, ADZ08740; YP_003356312, YP_003356313; ADL58895, ADL58894, ADL58283, ADL58893, ADL57751, ADL57749, ADL57750, ADL57748; CAA66401, CAA61212, CAA66400, CAA66402; CAA61213, CAA61214, CAA61210, CAA61211, CAA61209; YP_004444030; YP_002547540; YP_004511613; YP_004370144, AEB08963; YP_003051278; YP_003048298; ADL29297; YP_001046285, YP_001046287, YP_001046533; AET63761, AET64650, AET65189, AET64652; ABC56660, ABC56659, YP_447302, ABC56661, ABC56658, ABC56657;
83: Formylmethanofuran dehydrogenase (Fmd) (Fd ²⁻)	<i>Methanothermobacter thermotrophicus</i> ; <i>Methanothermobacter thermotrophicus</i> ; <i>Agrobacterium</i> sp. H13-3; <i>Agrobacterium vitis</i> S4; <i>Methylomonas methanica</i> MC09; <i>Desulfobacca acetoxidans</i> DSM 11109; <i>Methylovorus glucosetrophus</i> SIP3-4; <i>Methylotenera mobilis</i> JLW8; <i>Methylotenera versatilis</i> 301; <i>Methanoculleus marisnigri</i> JR1; <i>Methanosaeta harundinacea</i> 6Ac; <i>Methanosphaera stadtmanae</i> ;	YP_003356312, YP_003356313; ADL58895, ADL58894, ADL58283, ADL58893, ADL57751, ADL57749, ADL57750, ADL57748; CAA66401, CAA61212, CAA66400, CAA66402; CAA61213, CAA61214, CAA61210, CAA61211, CAA61209; YP_004444030; YP_002547540; YP_004511613; YP_004370144, AEB08963; YP_003051278; YP_003048298; ADL29297; YP_001046285, YP_001046287, YP_001046533; AET63761, AET64650, AET65189, AET64652; ABC56660, ABC56659, YP_447302, ABC56661, ABC56658, ABC56657;
84: Formyl transferase	<i>Methanothermobacter marburgensis</i> str. Marburg; <i>Methanosaeta harundinacea</i> 6Ac; <i>Methanosarcina barkeri</i> ; <i>Methanopyrus kandleri</i> AV19; <i>Thermosiphon melanesiensis</i> BI429; <i>Desulfobacca acetoxidans</i> DSM 11109; <i>Methylbacterium chloromethanicum</i> ; <i>Methylomicrobium alcaliphilum</i> ;	ADL59225, YP_003850538; AET65566; CAA62582; NP_614099; YP_001305762; YP_004369335; YP_002421530; YP_004917963;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
85: 5,10-Methenyl-tetrahydromethanopterin (H4 methanopterin) cyclohydrolase	<i>Methanopyrus kandleri</i> AV19;	NP_613403;
	<i>Methanoculleus marisnigri</i> JR1;	YP_001046543;
	<i>Methanocorpusculum labreanum</i> Z;	YP_001029658, YP_001029834;
	<i>Methanopyrus kandleri</i> AV19;	AAM02029, AAM01333;
	<i>Methanocella paludicola</i> SANAE;	YP_003356088, BAI61105;
	<i>Methanosphaera stadtmanae</i> ;	ABC57615, YP_448258;
	<i>Methanothermobacter feravidus</i> DSM 2088;	YP_004003819;
	<i>Methanosalsum zhilinae</i> DSM 4017;	AEH61193;
	<i>Methanohalophilus mahii</i> DSM 5219;	ADE36644;
	<i>Methanoplanus petrolearius</i> ;	ADN34846;
	<i>Archaeoglobus veneficus</i> SNP6;	YP_004342719;
	<i>Planctomyces brasiliensis</i> DSM 5305;	YP_004269775;
	<i>Methylobacillus flagellates</i> ;	AAD55893;
	<i>Xanthobacter autotrophicus</i> ;	AAD55896;
	<i>Methylosinus trichosporium</i> OB3b;	AAD56174;
	<i>Methylobacterium organophilum</i> ;	AAD55900;
	<i>Methylococcus capsulatus</i> ;	AAD55899;
	<i>Methylomicrobium kenyense</i> ;	AAS88982;
	<i>Methylomonas</i> sp. LW13;	AAS88987;
	<i>Methylosinus</i> sp. LW2;	AAS88975;
	<i>Methylomicrobium kenyense</i> ;	AAS86344;
	<i>Methanohalophilus mahii</i> DSM 5219;	YP_003542289;
	<i>Methanolinea tarda</i> NOBI-1;	EHF09908;
	<i>Methanothermococcus okinawensis</i> IH1;	YP_004577331;
	<i>Methanobacterium</i> sp. SWAN-1;	YP_004519292;
	<i>Methylomonas methanica</i> MC09;	YP_004513168;
	<i>Methanothermobacter marburgensis</i> ;	ADL57660, YP_003848973;
	<i>Methanosphaera stadtmanae</i> ;	YP_447224;
	<i>Methanococcus maripaludis</i> X1;	AEK19019;
	<i>Methanothermobacter thermotrophicus</i> ;	CAA63376;
	<i>Methanopyrus kandleri</i> ;	CAA43127;
	<i>Methylobacterium extorquens</i> AM1;	AAC27020;
	<i>Methylobacillus flagellatus</i> KT;	ABE49928;
<i>Xanthobacter autotrophicus</i> ;	AAD55895;	
<i>Methyloversatilis universalis</i> FAM5;	ZP_08504846;	
<i>Methylobacterium chloromethanicum</i> ;	ACK83011;	
<i>Methylobacterium populi</i> BJ001;	YP_001924478;	
<i>Methylobacterium extorquens</i> PA1;	YP_001639299;	
<i>Burkholderia</i> sp. CCGE1001;	YP_004230417;	
<i>Methylovorus</i> sp. MP688;	YP_004039958;	
<i>Methanocaldococcus fervens</i> AG86;	YP_003128308;	
<i>Methanocaldococcus jannaschii</i> ;	NP_247770;	
<i>Methanobrevibacter smithii</i> ;	YP_001273145;	
<i>Methanoplanus petrolearius</i> ;	ADN36752;	
<i>Methanocaldococcus</i> sp. FS406-22;	YP_003458803;	
<i>Methanocaldococcus infernus</i> ME;	ADG13507;	
<i>Methanocaldococcus fervens</i> AG86;	ACV24808;	
<i>anococcus maripaludis</i> C6;	ABX01642;	
<i>Stenotrophomonas</i> sp. SKA14;	EED39154, ZP_05135093;	
<i>Amycolatopsis mediterranei</i> S699;	AEK43785;	
<i>Corynebacterium glutamicum</i> ;	EHE83474;	
<i>Acinetobacter</i> sp. DR1;	ADI90167;	
<i>Acinetobacter baumannii</i> ABNIH4;	EGU03459;	
<i>Acinetobacter</i> sp. DR1;	YP_003731540;	
<i>Paenibacillus terrae</i> HPL-003;	AET61191;	
<i>Acinetobacter baumannii</i> ABNIH3;	EGT94264;	
<i>Cupriavidus necator</i> N-1;	AEI79563;	
<i>Herbaspirillum seropedicae</i> SmR1;	YP_003777169;	
<i>Burkholderia cenocepacia</i> HI2424;	YP_840196;	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423269, ADC46377;	
<i>Methanococcus voltae</i> A3;	ADL37005;	
<i>Methanococcus aeolicus</i> Nankai-3;	ABR56603;	
<i>Methanocaldococcus vulcanius</i> M7;	ACX71899;	
<i>Methanothermobacter marburgensis</i> ;	MTBMA_c02920;	
<i>Methanothermobacter marburgensis</i> str. Marburg;	ADL57900;	
88: Methyl-H4-methanopterin: corrinoid iron-sulfur protein methyltransferase		

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
95: [NiFe]-hydrogenase MvhADG (non-F420 reducing hydrogenase; methyl viologen-reducing hydrogenase)	<i>Methanosaeta harundinacea</i> 6Ac;	AET64165 (E);
	<i>Methanopyrus kandleri</i> AV19;	NP_613552 (hdrA);
	<i>Methanopyrus kandleri</i> AV19;	NP_613857 (hdrB);
	<i>Methanopyrus kandleri</i> AV19;	NP_613858 (hdrC);
	<i>Methanosphaera stadtmanae</i> ;	ABC56726 (mvhA);
	<i>Cyanobium</i> sp. PCC 7001;	EDY38497 (mvhA);
	<i>Methanothermobacter marburgensis</i> ;	ADL59096 (mvhA)
	<i>Methanobrevibacter ruminantium</i> M1	YP_003424648 (mvhA);
	<i>Desulfobacterium autotrophicum</i> HRM2	YP_002602450 (mvhA)
	<i>Desulfatibacillum alkenivorans</i> AK-01	ACL06634 (mvhA);
	<i>Methanothermobacter marburgensis</i> ;	ADL59095 (mvhB);
	<i>Desulfatibacillum alkenivorans</i> AK-01;	ACL06636 (mvhB);
	<i>Methanobrevibacter smithii</i> DSM 2374;	ZP_05975561 (mvhB);
	<i>Methanothermobacter marburgensis</i> ;	ADL59098 (mvhD);
	<i>Methanothermobacter marburgensis</i> ;	YP_003850411 (mvhD);
	<i>Methanobrevibacter smithii</i> ;	YP_001273574 (mvhD);
	<i>Methanobrevibacter smithii</i> ;	ABQ87206 (mvhD);
	<i>Methanothermobacter thermautotrophicus</i> ;	AAB02349 (mvhD);
	<i>Methanothermobacter marburgensis</i> ;	ADL59097 (mvhG);
	<i>Desulfatibacillum alkenivorans</i> AK-01;	ACL06635 (mvhG);
	<i>Cyanobium</i> sp. PCC 7001;	EDY38425 (mvhG);
	<i>Methanosphaera stadtmanae</i> ;	ABC56725 (mvhG);
	<i>Methanobrevibacter smithii</i> DSM 2374;	EFC93226 (mvhG);
	<i>Desulfatibacillum alkenivorans</i> AK-01;	ACL06638;
	<i>Desulfatibacillum alkenivorans</i> AK-01;	ACL03322;
	<i>Methanoculleus marisnigri</i> JR1;	YP_001046332 (hypF);
	<i>Methanocella paludicola</i> SANAE;	YP_003357229 (frhB-1);
	<i>Methanocella paludicola</i> SANAE;	YP_003357467 (frhB-2);
	<i>Methanocella paludicola</i> SANAE;	YP_003357509 (frhB-3);
	<i>Synechococcus elongatus</i> PCC 7942;	ABB57389 (frhB);
	<i>Synechocystis</i> sp. PCC 6803;	BAA18574, YP_001735870;
	<i>Synechococcus</i> sp. WH 7803;	YP_001225273;
	<i>Synechococcus</i> sp. RCC307;	YP_001227030;
<i>Cyanothece</i> sp. PCC 8802;	ACV00312 (frhB);	
<i>Cyanobium</i> sp. PCC 7001;	EDY39891 (fehB);	
<i>Synechococcus</i> sp. RS9916;	EAU74116 (frhB);	
<i>Synechococcus</i> sp. JA-2-3B'a(2-13);	YP_477499;	
<i>Pelotomaculum thermopropionicum</i> SI;	YP_001212042, YP_001211959;	
<i>Methanothermobacter fervidus</i> DSM 2088;	YP_004004590;	
<i>Methanococcus maripaludis</i> S2;	CAF30376 (A), NP_988502 (A);	
<i>Methanococcus maripaludis</i> S2;	NP_988505 (B);	
<i>Methanococcus maripaludis</i> S2;	NP_988503 (D);	
<i>Methanococcus maripaludis</i> S2;	NP_988504 (G);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423444 (ahaA);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423445 (ahaB);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423442 (ahaC);	
<i>Methanobrevibacter ruminantium</i> M1	ADC46554 (ahaD);	
<i>Methanobrevibacter ruminantium</i> M1;	ADC46549 (ahaE);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423443 (ahaF);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423438 (ahaH)	
<i>Methanobrevibacter ruminantium</i> M1;	ADC46547 (ahaI);	
<i>Methanobrevibacter ruminantium</i> M1;	YP_003423440 (ahaK);	
<i>Ferroplasma acidarmanus fer1</i> ;	ZP_05570724;	
<i>Thermococcus sibiricus</i> MM 739;	YP_002995194;	
<i>Thermoproteus tenax</i> Kra 1;	CCC82573;	
<i>Thermoproteus tenax</i> Kra 1;	CCC82176;	
<i>Methanosarcina mazei</i> Go1;	AAC06375 (ahaA);	
<i>Methanosarcina mazei</i> Go1;	AAC06376 (ahaB);	
<i>Methanosarcina mazei</i> Go1;	AAC06373 (ahaC);	
<i>Methanosarcina mazei</i> Go1;	AAC06377 (ahaD)	
<i>Methanosarcina mazei</i> Go1;	AAC06372 (ahaE);	
<i>Methanosarcina mazei</i> Go1;	AAC06374 (ahaF);	
<i>Methanosarcina mazei</i> Go1;	AAC06378 (ahaG);	
<i>Methanosarcina mazei</i> Go1;	CAA58177 (mhtA);	
<i>Methanosarcina acetivorans</i> C2A;	NP_616088 (mhtA);	
<i>Archaeoglobus fulgidus</i> DSM 4304;	NP_070209 (mhtA);	
<i>Ferroglobus placidus</i> DSM 10642;	ADC65001 (mhtA);	
<i>Methanosarcina acetivorans</i> C2A;	NP_616088 (mhtB);	
<i>Archaeoglobus fulgidus</i> DSM 4304;	NP_070209 (mhtB);	
98: Membrane bound cytochrome-containing F420- nonreducing hydrogenase (VhtGAC, VhtD)	<i>Methanosarcina acetivorans</i> C2A;	NP_616088 (mhtA);
	<i>Archaeoglobus fulgidus</i> DSM 4304;	NP_070209 (mhtA);
	<i>Ferroglobus placidus</i> DSM 10642;	ADC65001 (mhtA);

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
100: Pyridoxal phosphate-dependent L-tyrosine decarboxylase (mfnA for methanofuran synthesis)	<i>Cyanothece</i> sp. ATCC 51472;	EHC24992 (cofH);
	<i>Methanospaera stadmanae</i> ;	ABC56793 (cofH);
	<i>Methanococcus maripaludis</i> S2;	NP_987177 (cofH);
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424008 (cofH);
	<i>Methanosarcina mazei</i> Go1;	NP_634520 (cofH);
	<i>Methanocella paludicola</i> SANAE;	YP_003357511 (cofH);
	<i>Methanocella paludicola</i> SANAE;	YP_003355454;
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424638;
	<i>Thermococcus gammatolerans</i> EJ3;	YP_002960503;
	<i>Halobacterium salinarum</i> R1;	YP_001688512;
101a: MptA: GTP cyclohydrolase (for Methanopterin synthesis)	<i>Methanothermobacter marburgensis</i> ;	ADL59079;
	<i>Thermococcus gammatolerans</i> EJ3;	ACS34639;
	<i>Haloferax volcanii</i> DS2;	YP_003534871;
	<i>Methanospaera stadmanae</i> ;	YP_447347;
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424704;
	<i>Methanococcus maripaludis</i> S2;	NP_987154;
	<i>Pyrococcus horikoshii</i> OT3;	NP_143623;
	<i>Thermococcus gammatolerans</i> EJ3;	YP_002959796;
	<i>Methanosarcina mazei</i> Go1;	NP_633246;
	<i>Methanospirillum hungatei</i> JF-1;	YP_503757;
101b: MptB: Cyclic phosphodiesterase (for Methanopterin synthesis)	<i>Thermococcus kodakarensis</i> KOD1;	YP_183206;
	<i>Methanopyrus kandleri</i> AV19;	NP_613770;
	<i>Methanosarcina acetivorans</i> C2A;	NP_619377;
	<i>Methanocaldococcus fervens</i> AG86;	YP_003128348;
	<i>Methanoregula boonei</i> 6A8;	YP_001403641;
	<i>Methanothermobacter thermautotrophicus</i> ;	NP_276324;
	<i>Methanosarcina barkeri</i> str. Fusaro;	YP_304731;
	<i>Methanocaldococcus jannaschii</i> ;	NP_247760;
	<i>Methanococcus maripaludis</i> C5;	ABO35741;
	<i>Roseobacter denitrificans</i> OCh 114;	YP_683148;
101c: RFAP: Ribofuranosylaminobenzene 5'-phosphate synthase (for Methanopterin synthesis)	<i>Arabidopsis thaliana</i> ;	AEE84108;
	<i>Zea mays</i> ;	NP_001151923;
	<i>Medicago truncatula</i> ;	XP_003629873;
	<i>Methanothermus fervidus</i> DSM 2088;	YP_004003771;
	<i>Methanocella paludicola</i> SANAE;	YP_003356610;
	<i>Methanoplanus petrolearius</i> ;	ADN37264;
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424432;
	<i>Archaeoglobus veneficus</i> SNP6;	YP_004342012;
	<i>Thermococcus</i> sp. AM4;	YP_002582695;
	<i>Methanococcus maripaludis</i> S2;	NP_987399;
102a: ComA: Phosphosulfolactate synthase (for Coenzyme M synthesis)	<i>Methanothermus fervidus</i> DSM 2088;	ADP77009;
	<i>Methanocella paludicola</i> SANAE;	BAI61627;
	<i>Methanothermobacter marburgensis</i> ;	ADL57861;
	<i>Methanococcus maripaludis</i> S2;	NP_987393;
	<i>Methanospaera stadmanae</i> ;	ABC57647;
	<i>Methanothermus fervidus</i> DSM 2088;	YP_004004617;
	<i>Methanothermococcus okinawensis</i> IH1;	YP_004575938;
	<i>Methanobacterium</i> sp. SWAN-1;	YP_004519242;
	<i>Methanocaldococcus fervens</i> AG86;	YP_003127444;
	<i>Methanococcus voltae</i> A3;	ADI36986;
102b: ComB: 2-Phosphosulfolactate phosphatase (for Coenzyme M synthesis)	<i>Methanococcus maripaludis</i> C6;	YP_001548728;
	<i>Methanobacterium</i> sp. AL-21;	YP_004291430;
	<i>Methanococcus aeolicus</i> Nankai-3;	YP_001324357;
	<i>Methanoterris igneus</i> Kol 5;	AEF96400;
	<i>Methanobacterium</i> sp. AL-21	ADZ10458;
	<i>Methanococcus maripaludis</i> X1;	AEK19167;
	<i>Methanocaldococcus infernus</i> ME;	ADG13665;
	<i>Methanocaldococcus</i> sp. FS406-22;	YP_003457919;
	<i>Methanococcus maripaludis</i> S2;	NP_987281;
	<i>Methanopyrus kandleri</i> AV19;	AAM01355;
	<i>Methanothermobacter marburgensis</i> ;	YP_003850451;
	<i>Methanococcus maripaludis</i> S2;	CAF29717;
	<i>Methanocella paludicola</i> SANAE;	YP_003357619;
	<i>Methanothermus fervidus</i> DSM 2088;	YP_004004784;
	<i>Methanothermus fervidus</i> DSM 2088;	ADP78022;
	<i>Methanobacterium</i> sp. AL-21;	YP_004289567;
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424691;
	<i>Synechocystis</i> sp. PCC 6803;	BAK50080;
	<i>Synechococcus</i> sp. JA-2-3B'a(2-13);	YP_476548;

TABLE 1-continued

lists examples of enzymes for construction of designer Calvin-cycle-linked pathways for production of butanol and related higher alcohols.		
Enzyme/callout number	Source (Organism)	GenBank Accession Number, JGI Protein ID or Citation
102c: ComC: Sulfolactate dehydrogenase (for Coenzyme M synthesis)	<i>Synechococcus</i> sp. PCC 7002;	YP_001735079;
	<i>Synechococcus</i> sp. WH 7803;	YP_001224757;
	<i>Cyanothece</i> sp. ATCC 51472;	EHC21417;
	<i>Synechococcus</i> sp. WH 8016;	ZP_08955317;
	<i>Methanothermobacter marburgensis</i> ;	ADL59162;
	<i>Methanosphaera stadtmanae</i> ;	ABC56689;
	<i>Methanothermobacter marburgensis</i> ;	YP_003850475;
	<i>Methanothermobacter fervidus</i> DSM 2088;	YP_004003953;
	<i>Roseobacter litoralis</i> Och 149;	YP_004689622;
	<i>Methanococcus maripaludis</i> C5;	ABO34766;
102d: ComDE: Sulfofynivate decarboxylase (for Coenzyme M synthesis)	<i>Methanothermobacter fervidus</i> DSM 2088;	ADP77191;
	<i>Methanosarcina acetivorans</i> C2A;	NP_618188;
	<i>Methanocella paludicola</i> SANAE;	YP_003357048;
	<i>Methanocorpusculum labreanum</i> Z;	YP_001029945;
	<i>Methanoculleus marisnigri</i> JR1;	ABN56047;
	<i>Methanosarcina barkeri</i> str. Fusaro;	YP_306991;
	<i>Methanocella paludicola</i> SANAE;	BAI62065;
	<i>Methanosphaera stadtmanae</i> ;	ABC56687;
	<i>Methanococcus maripaludis</i> S2;	NP_988809;
	<i>Methanothermobacter marburgensis</i> ;	comF;
102e: ComF: Sulfoacetaldehyde dehydrogenase (for Coenzyme M synthesis)	<i>Methanothermobacter marburgensis</i> ;	comF;
	<i>Methanothermobacter thermautotrophicus</i>	comF;
103a: LeuA homolog: Isopropylmalate synthase (for Coenzyme B synthesis)	<i>Methanopyrus kandleri</i> AV19;	AAM01606;
	<i>Methanothermobacter thermautotrophicus</i> ;	AAB85956;
	<i>Thermoproteus tenax</i> ;	CAF18516;
	<i>Thermoplasma volcanium</i> GSS1;	NP_111428;
	<i>Methanobrevibacter smithii</i> ;	ABQ87451;
	<i>Methanosphaera stadtmanae</i> ;	YP_447259;
	<i>Methanobrevibacter ruminantium</i> M1;	YP_003424897;
	<i>Methanococcus maripaludis</i> S2;	NP_988183;
	<i>Synechocystis</i> sp. PCC 6803	NP_442009;
	<i>Synechococcus elongatus</i> PCC 7942;	ABB56460;
103b: LeuB homolog: Isopropylmalate dehydrogenase (for Coenzyme B synthesis)	<i>Cyanothece</i> sp. ATCC 51472;	EHC25498;
	<i>Synechococcus</i> sp. WH 8016;	ZP_08954784;
	<i>Synechococcus</i> sp. JA-2-3B'a(2-13)	YP_477672;
	<i>Thermosynechococcus elongatus</i> BP-1;	NP_682187;
	<i>Methanopyrus kandleri</i> AV19;	NP_614498;
	<i>Methanothermobacter marburgensis</i> ;	ADL58232;
	<i>Methanothermobacter fervidus</i> DSM 2088;	YP_004004146;
	<i>Methanocella paludicola</i> SANAE;	YP_003358048;
	<i>Methanosphaera stadtmanae</i> ;	YP_447715;
	<i>Methanocella paludicola</i> SANAE;	BAI63065;
103c: LeuCD homolog: Isopropylmalate isomerase (for Coenzyme B synthesis)	<i>Methanococcus maripaludis</i> S2;	CAF30095;
	<i>Synechocystis</i> sp. PCC 6803;	NP_441348;
	<i>Synechococcus elongatus</i> PCC 7942;	ABB57535;
	<i>Cyanothece</i> sp. ATCC 51472;	EHC23198;
	<i>Synechococcus</i> sp. JA-2-3B'a(2-13);	YP_477855;
	<i>Thermosynechococcus elongatus</i> BP-1;	NP_682390;
	<i>Marinobacter adhaerens</i> HP15;	ADP98363, ADP98362;
	<i>Halorhabdus tiamatea</i> SARL4B;	ZP_08559069;
	<i>Haloarcula marismortui</i> ATCC 43049;	YP_135090;
	<i>Halomicrobium mukohataei</i> ;	YP_003178469;
	<i>Haladaptatus paucihalophilus</i> DX253;	ZP_08045715;
	<i>Escherichia coli</i> O103:H2 str. 12009;	YP_003220086, YP_003220085;
	<i>Synechocystis</i> sp. PCC 6803;	NP_442926, NP_441618;
	<i>Cyanothece</i> sp. PCC 8801;	YP_002370476, YP_002373868;
	<i>Nostoc</i> sp. PCC 7120;	NP_485460, NP_485459;
	<i>Synechococcus</i> sp. JA-2-3B'a(2-13);	YP_478232, YP_476588;
	<i>Thermosynechococcus elongatus</i> BP-1;	NP_681699, NP_682024;

Designer Calvin-Cycle-Channeled 1-Butanol Producing Pathways

[0173] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglyc-

erate, and converts it into 1-butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-05, 36-43 in FIG. 4): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05,

citramalate synthase 36, 2-methylmalate dehydratase 37, 3-isopropylmalate dehydratase 38, 3-isopropylmalate dehydrogenase 39, 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 2-keto acid decarboxylase 42, and alcohol dehydrogenase (NAD dependent) 43. In this pathway design, as mentioned above, the NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 serve as a NADPH/NADH conversion mechanism that can convert certain amount of photosynthetically generated NADPH to NADH which can be used by the NADH-requiring alcohol dehydrogenase 43 (examples of its encoding gene with the following GenBank accession numbers: BAB59540, CAA89136, NP_148480) for production of 1-butanol by reduction of butyraldehyde.

[0174] According to one of the various embodiments, it is a preferred practice to also use an NADPH-dependent alcohol dehydrogenase 44 that can use NADPH as the source of reductant so that it can help alleviate the requirement of NADH supply for enhanced photobiological production of butanol and other alcohols. As listed in Table 1, examples of NADPH-dependent alcohol dehydrogenase 44 include (but not limited to) the enzyme with any of the following GenBank accession numbers: YP_001211038, ZP_04573952, XP_002494014, CAY71835, NP_417484, EFC99049, and ZP_02948287.

[0175] Note, the 2-keto acid decarboxylase 42 (e.g., AAS49166, ADA65057, CAG34226, AAA35267, CAA59953, A0QBE6, A0PL16) and alcohol dehydrogenase 43 (and/or 44) have quite broad substrate specificity. Consequently, their use can result in production of not only 1-butanol but also other alcohols such as propanol depending on the genetic and metabolic background of the host photosynthetic organisms. This is because all 2-keto acids can be converted to alcohols by the 2-keto acid decarboxylase 42 and alcohol dehydrogenase 43 (and/or 44) owing to their broad substrate specificity. Therefore, according to another embodiment, it is a preferred practice to use a substrate-specific enzyme such as butanol dehydrogenase 12 when/if production of 1-butanol is desirable. As listed in Table 1, examples of butanol dehydrogenase 12 are NADH-dependent butanol dehydrogenase (e.g., GenBank: YP_148778, NP_561774, AAG23613, ZP_05082669, ADO12118) and/or NAD(P)H-dependent butanol dehydrogenase (e.g., NP_562172, AAA83520, EFB77036, EFF67629, ZP_06597730, EFE12215, EFC98086, ZP_05979561).

[0176] In one of the various embodiments, another designer Calvin-cycle-channelled 1-butanol production pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 1-butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03, 04, 45-52 and 40-43 (44/12) in FIG. 4): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, phosphoenolpyruvate carboxylase 45, aspartate aminotransferase 46, aspartokinase 47, aspartate-semialdehyde dehydrogenase 48, homoserine dehydrogenase 49, homoserine kinase 50, threonine synthase 51, threonine ammonia-lyase 52, 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, 2-keto acid decarboxylase 42, and NAD-dependent alcohol dehydrogenase 43 (and/or NADPH-dependent alcohol dehydrogenase 44, or butanol dehydrogenase 12).

[0177] According to another embodiment, the amino-acids-metabolism-related 1-butanol production pathways [numerical labels 03-05, 36-43; and/or 03, 04, 45-52 and 39-43 (44/12)] can operate in combination and/or in parallel with other photobiological butanol production pathways. For example, as shown also in FIG. 4, the Fructose-6-phosphate-branched 1-butanol production pathway (numerical labels 13-32 and 44/12) can operate with the parts of amino-acids-metabolism-related pathways [numerical labels 36-42, and/or 45-52 and 40-42) with pyruvate and/or phosphoenolpyruvate as their joining points.

[0178] Examples of designer Calvin-cycle-channelled 1-butanol production pathway genes (DNA constructs) are shown in the DNA sequence listings. SEQ ID NOS: 58-70 represent a set of designer genes for a designer nirA-promoter-controlled Calvin-cycle-channelled 1-butanol production pathway (as shown with numerical labels 34, 35, 03-05, and 36-43 in FIG. 4) in a host oxyphotobacterium such as *Thermosynechococcus elongatus* BP1. Briefly, SEQ ID NO: 58 presents example 58 of a designer nirA-promoter-controlled NADPH-dependent Glyceraldehyde-3-Phosphate Dehydrogenase (34) DNA construct (1417 bp) that comprises: a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1277) selected/modified from the sequences of a *Staphylococcus aureus* 04-02981 NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase (GenBank: ADC37857), a 120-bp rbcS terminator from BP1 (1278-1397), and a PCR RE primer (1398-1417) at the 3' end.

[0179] SEQ ID NO: 59 presents example 59 of a designer nirA-promoter-controlled NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (35) DNA construct (1387 bp) that comprises: a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1247) selected/modified from the sequences of an *Edwardsiella tarda* FL6-60 NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GenBank: ADM41489), a 120-bp rbcS terminator from BP1 (1248-1367), and a PCR RE primer (1368-1387) at the 3' end.

[0180] SEQ ID NO: 60 presents example 60 of a designer nirA-promoter-controlled Phosphoglycerate Mutase (03) DNA construct (1627 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1487) selected/modified from the sequences of a *Oceanithermus profundus* DSM 14977 phosphoglycerate mutase (GenBank: ADR35708), a 120-bp rbcS terminator from BP1 (1488-1607), and a PCR RE primer (1608-1627).

[0181] SEQ ID NO: 61 presents example 61 of a designer nirA-promoter-controlled Enolase (04) DNA construct (1678 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1538) selected from the sequences of a *Syntrophothermus* Enolase (GenBank: ADI02602), a 120-bp rbcS terminator from BP1 (1539-1658), and a PCR RE primer (1659-1678).

[0182] SEQ ID NO: 62 presents example 62 of a designer nirA-promoter-controlled Pyruvate Kinase (05) DNA construct (2137 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1997) selected from the sequences of a *Syntrophother-*

mus lipocalidus pyruvate kinase (GenBank: ADI02459), a 120-bp *rbcS* terminator from BP1 (1998-2117), and a PCR RE primer (2118-2137).

[0183] SEQ ID NO: 63 presents example 63 of a designer *nirA*-promoter-controlled Citramalate Synthase (36) DNA construct (2163 bp) that includes a PCR FD primer (sequence 1-20), a 305-bp *nirA* promoter (21-325), an enzyme-encoding sequence (326-1909) selected and modified from *Hydrogenobacter thermophilus* TK-6 citramalate synthase (YP_003433013), a 234-bp *rbcS* terminator from BP1 (1910-2143), and a PCR RE primer (2144-2163).

[0184] SEQ ID NO: 64 presents example 64 of a designer *nirA*-promoter-controlled 3-Isopropylmalate/(R)-2-Methylmalate Dehydratase (37) DNA construct (2878 bp) consisting of a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), a 3-isopropylmalate/(R)-2-methylmalate dehydratase large subunit-encoding sequence (252-2012) selected/modified from the sequences of an *Eubacterium* 3-isopropylmalate/(R)-2-methylmalate dehydratase large subunit (YP_002930810), a 231-bp *nirA* promoter from *Thermosynechococcus* (2013-2243), a 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit-encoding sequence (2244-2738) selected/modified from the sequences of an *Eubacterium* 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit (YP_002930809), a 120-bp *rbcS* terminator from BP1 (2739-2858), and a PCR RE primer (2859-2878).

[0185] SEQ ID NO: 65 presents example 65 of a designer *nirA*-promoter-controlled 3-Isopropylmalate Dehydratase (38) DNA construct (2380 bp) comprises: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), a 3-isopropylmalate dehydratase large subunit-encoding sequence (252-1508) selected/modified from the sequences of a *Thermotoga petrophila* 3-isopropylmalate dehydratase large subunit (ABQ46641), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (1509-1739), a 3-isopropylmalate dehydratase small subunit-encoding sequence (1740-2240) selected/modified from the sequences of a *Thermotoga* 3-isopropylmalate dehydratase small subunit (ABQ46640), a 120-bp *rbcS* terminator from BP1 (2241-2360), and a PCR RE primer (2361-2380).

[0186] SEQ ID NO: 66 presents example 66 of a designer *nirA*-promoter-controlled 3-Isopropylmalate Dehydrogenase (39) DNA construct (1456 bp) consisting of: a PCR FD primer (1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), a 3-isopropylmalate dehydrogenase-encoding sequence (252-1316) selected from the sequences of a *Thermotoga* 3-isopropylmalate dehydrogenase (GenBank: CP000702 Region 349983 . . . 351047), a 120-bp *rbcS* terminator from BP1 (1317-1436), and a PCR RE primer (1437-1456).

[0187] SEQ ID NO: 67 presents example 67 of a designer *nirA*-promoter-controlled 2-Isopropylmalate Synthase (40, EC 4.1.3.12) DNA construct (1933 bp) consisting of: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* (21-251), an enzyme-encoding sequence (252-1793) selected/modified from the sequences of a *Thermotoga petrophila* 3-isopropylmalate dehydrogenase (CP000702 Region: 352811 . . . 354352), a 120-bp *rbcS* terminator from BP1 (1794-1913), and a PCR RE primer (1914-1933).

[0188] SEQ ID NO: 68 presents example 68 of a designer *nirA*-promoter-controlled Isopropylmalate Isomerase (41) DNA construct (2632 bp) comprises: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), a isopropylmalate isomerase large subunit-encoding sequence (252-1667) selected/modified from the sequences of a *Geobacillus kaustophilus* 3-isopropylmalate isomerase large subunit (YP_148509), a 231-bp *nirA* promoter from *Thermosynechococcus* (1668-1898), a isopropylmalate isomerase small subunit-encoding sequence (1899-2492) selected from the sequences of a *Geobacillus kaustophilus* isopropylmalate isomerase small subunit (YP_148508), a 120-bp *rbcS* terminator from BP1 (2493-2612), and a PCR RE primer (2613-2632).

[0189] SEQ ID NO: 69 presents example 69 of a designer *nirA*-promoter-controlled 2-Keto Acid Decarboxylase (42) DNA construct (2035 bp) consisting of: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), a 2-keto acid decarboxylase-encoding sequence (252-1895) selected/modified from the sequences of a *Lactococcus lactis* branched-chain alpha-ketoacid decarboxylase (AAS49166), a 120-bp *rbcS* terminator from BP1 (1896-2015), and a PCR RE primer (2016-2035) at the 3' end.

[0190] SEQ ID NO: 70 presents example 70 of a designer *nirA*-promoter-controlled NAD-dependent Alcohol Dehydrogenase (43) DNA construct (1426 bp) consisting of: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1286) selected/modified from the sequences of an *Aeropyrum pernix* K1 NAD-dependent alcohol dehydrogenase (NP_148480), a 120-bp *rbcS* terminator from BP1 (1287-1406), and a PCR RE primer (1407-1426).

[0191] As mentioned before, use of an NADPH-dependent alcohol dehydrogenase 44 that can use NADPH as the source of reductant can help alleviate the requirement of NADH supply for enhanced photobiological production of butanol and other alcohols. SEQ ID NO: 71 presents example 71 of a designer *nirA*-promoter-controlled NADPH-dependent Alcohol Dehydrogenase (44) DNA construct (1468 bp) that comprises: a PCR FD primer (sequence 1-20), a 231-bp *nirA* promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1328) selected from the sequences of a *Pichia pastoris* NADPH-dependent medium chain alcohol dehydrogenase with broad substrate specificity (XP_002494014), a 120-bp *rbcS* terminator from BP1 (1329-1458), and a PCR RE primer (1459-1468) at the 3' end. In one of the examples, this type of NADPH-dependent alcohol dehydrogenase gene (SEQ ID NO: 71) is also used in construction of Calvin-cycle-channeled butanol production pathway.

[0192] However, because of the broad substrate specificity of the 2-keto acid decarboxylase (42, SEQ ID NO: 69) and the alcohol dehydrogenase (43, SEQ ID NO: 70; or 44, SEQ ID NO: 71), the pathway expressed with designer genes of SEQ ID NO: 69 and SEQ ID NO: 71 (and/or SEQ ID NO: 70) can result in the production of alcohol mixtures rather than single alcohols since all 2-keto acids can be converted to alcohols by the two broad substrate specificity enzymes. Therefore, to improve the specificity for 1-butanol production, it is a preferred practice to use a more substrate-specific butanol dehydrogenase 12. SEQ ID NO: 72 presents example 72 of a designer *nirA*-promoter-controlled NADPH-dependent

Butanol Dehydrogenase (12a) DNA construct (1555 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1415) selected/modified from the sequences of a *Geobacillus kaustophilus* NADH-dependent butanol dehydrogenase (YP_148778), a 120-bp rbcS terminator from BP1 (1416-1535), and a PCR RE primer (1536-1555) at the 3' end.

[0193] SEQ ID NO: 73 presents example 73 of a designer nirA-promoter-controlled NADPH-dependent Butanol Dehydrogenase (12b) DNA construct (1558 bp) consisting of a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), a NADPH-dependent butanol dehydrogenase-encoding sequence (252-1418) selected/modified from the sequences of a *Clostridium perfringens* NADPH-dependent butanol dehydrogenase (NP_562172), a 120-bp rbcS terminator from BP1 (1419-1528), and a PCR RE primer (1529-1558) at the 3' end.

[0194] Use of SEQ ID NOS: 72 and/or 73 (12a and/or 12b) along with SEQ ID NOS: 58-69 represents a specific Calvin-cycle-channeled 1-butanol production pathway numerically labeled as 34, 35, 03-05, 36-42 and 12 in FIG. 4.

[0195] SEQ ID NOS: 74-81 represent an alternative (amino acids metabolism-related) pathway (45-52 in FIG. 4) that branches from the point of phosphoenolpyruvate and merges at the point of 2-ketobutyrate in the Calvin-cycle-channeled 1-butanol production pathway. Briefly, SEQ ID NO: 74 presents example 74 of a designer nirA-promoter-controlled Phosphoenolpyruvate Carboxylase (45) DNA construct (3646 bp) consisting of: a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-3506) selected/modified from the sequences of a *Thermaerobacter subterraneus* DSM 13965 Phosphoenolpyruvate carboxylase (EFR61439), a 120-bp rbcS terminator from BP1 (3507-3626), and a PCR RE primer (3627-3646) at the 3' end.

[0196] SEQ ID NO: 75 presents example 75 of a designer nirA-promoter-controlled Aspartate Aminotransferase (46) DNA construct (1591 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1451) selected/modified from the sequences of a *Thermotoga lettingae* aspartate aminotransferase (YP_001470126), a 120-bp rbcS terminator from BP1 (1452-1471), and a PCR RE primer (1472-1591).

[0197] SEQ ID NO: 76 presents example 76 of a designer nirA-promoter-controlled Aspartate

[0198] Kinase (47) DNA construct (1588 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1448) selected/modified from the sequences of a *Thermotoga lettingae* TMO aspartate kinase (YP_001470361), a 120-bp rbcS terminator from BP1 (1449-1568), and a PCR RE primer (1569-1588).

[0199] SEQ ID NO: 77 presents example 77 of a designer nirA-promoter-controlled Aspartate-Semialdehyde Dehydrogenase (48) DNA construct (1411 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1271) selected/modified from the sequences of a *Thermotoga lettingae* TMO aspartate-semial-

dehyde dehydrogenase (YP_001470981), a 120-bp rbcS terminator from BP1 (1272-1391), and a PCR RE primer (1392-1411) at the 3' end.

[0200] SEQ ID NO: 78 presents example 78 of a designer nirA-promoter-controlled Homoserine Dehydrogenase (49) DNA construct (1684 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1544) selected/modified from the sequences of a *Syntrophothermus lipocalidus* DSM 12680 homoserine dehydrogenase (ADI02231), a 120-bp rbcS terminator from BP1 (1545-1664), and a PCR RE primer (1665-1684) at the 3' end.

[0201] SEQ ID NO: 79 presents example 79 of a designer nirA-promoter-controlled Homoserine Kinase (50) DNA construct (1237 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1097) selected/modified from the sequences of a *Thermotoga petrophila* RKU-1 Homoserine Kinase (YP_001243979), a 120-bp rbcS terminator from BP1 (1098-1217), and a PCR RE primer (1218-1237) at the 3' end.

[0202] SEQ ID NO: 80 presents example 80 of a designer nirA-promoter-controlled Threonine Synthase (51) DNA construct (1438 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1298) selected from the sequences of a *Thermotoga* Threonine Synthase (YP_001243978), a 120-bp rbcS terminator from BP1 (1299-1418), and a PCR RE primer (1419-1438).

[0203] SEQ ID NO: 81 presents example 81 of a designer nirA-promoter-controlled Threonine Ammonia-Lyase (52) DNA construct (1600 bp) consisting of a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1460) selected/modified from the sequences of a *Geobacillus kaustophilus* threonine ammonia-lyase (BAD75876), a 120-bp rbcS terminator from BP1 (1461-1580), and a PCR RE primer (1581-1600) at the 3' end.

[0204] Note, SEQ ID NOS: 58-61, 74-81, 66-69, and 72 (and/or 73) represent a set of sample designer genes that can express a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 1-butanol production pathway of 34, 35, 03, 04, 45-52 40, 41, 39, 42, and 12 while SEQ ID NOS: 58-69 and 72 (and/or 73) represent another set of sample designer genes that can express another Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 1-butanol production pathway as numerically labeled as 34, 35, 03-05, 36-42, and 12 in FIG. 4. The net results of the designer photosynthetic NADPH-enhanced pathways in working with the Calvin cycle are photobiological production of 1-butanol (CH₃CH₂CH₂CH₂OH) from carbon dioxide (CO₂) and water (H₂O) using photosynthetically generated ATP (Adenosine triphosphate) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) according to the following process reaction:



Designer Calvin-Cycle-Channeled 2-Methyl-1-Butanol Producing Pathways

[0205] According to one of the various embodiments, a designer Calvin-cycle-channeled 2-Methyl-1-Butanol production pathway is created that takes the Calvin-cycle inter-

mediate product, 3-phosphoglycerate, and converts it into 2-methyl-1-butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-05, 36-39, 53-55, 42, 43 or 44/56 in FIG. 5): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, citramalate synthase 36, 2-methylmalate dehydratase 37, 3-isopropylmalate dehydratase 38, 3-isopropylmalate dehydrogenase 39, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, 2-keto acid decarboxylase 42, and NAD-dependent alcohol dehydrogenase 43 (or NADPH-dependent alcohol dehydrogenase 44; more preferably, 2-methylbutyraldehyde reductase 56).

[0206] In another embodiment, a designer Calvin-cycle-channeled 2-methyl-1-butanol production pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into 2-methyl-1-butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03, 04, 45-55, 42, 43 or 44/56 in FIG. 5): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, phosphoenolpyruvate carboxylase 45, aspartate aminotransferase 46, aspartokinase 47, aspartate-semialdehyde dehydrogenase 48, homoserine dehydrogenase 49, homoserine kinase 50, threonine synthase 51, threonine ammonia-lyase 52, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, 2-keto acid decarboxylase 42, and NAD dependent alcohol dehydrogenase 43 (or NADPH dependent alcohol dehydrogenase 44; more preferably, 2-methylbutyraldehyde reductase 56).

[0207] These pathways (FIG. 5) are quite similar to those of FIG. 4, except that acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, and 2-methylbutyraldehyde reductase 56 are used to produce 2-Methyl-1-Butanol.

[0208] SEQ ID NO: 82 presents example 82 of a designer nirA-promoter-controlled Acetolactate Synthase (53) DNA construct (2107 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an acetolactate synthase-encoding sequence (252-1967) selected/modified from the sequences of a *Bacillus subtilis* subsp. *subtilis* str. 168 acetolactate synthase (CAB07802), a 120-bp rbcS terminator from BP1 (1968-2087), and a PCR RE primer (2088-2107) at the 3' end.

[0209] SEQ ID NO: 83 presents example 83 of a designer nirA-promoter-controlled Ketol-Acid Reductoisomerase (54) DNA construct (1405 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), a ketol-acid reductoisomerase-encoding sequence (252-1265) selected/modified from the sequences of a *Syntrophothermus lipocalidus* DSM 12680 ketol-acid reductoisomerase (ADI02902), a 120-bp rbcS terminator from BP1 (1266-1385), and a PCR RE primer (1386-1405) at the 3' end.

[0210] SEQ ID NO: 84 presents example 84 of a designer nirA-promoter-controlled Dihydroxy-Acid Dehydratase (55) DNA construct (2056 bp) that includes a PCR FD primer (1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1916) selected from the sequences of a *Thermotoga*

dihydroxy-acid dehydratase (YP_001243973), a 120-bp rbcS terminator from BP1 (1917-2036), and a PCR RE primer (2037-2056).

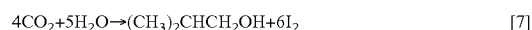
[0211] SEQ ID NO: 85 presents example 85 of a designer nirA-promoter-controlled 2-Methylbutyraldehyde Reductase (56) DNA construct (1360 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1220) selected/modified from the sequences of a *Schizosaccharomyces japonicus* 2-methylbutyraldehyde reductase (XP_002173231), a 120-bp rbcS terminator from BP1 (1221-1340), and a PCR RE primer (1341-1360) at the 3' end.

[0212] Note, SEQ ID NOS: 58-66, 82-84, 69 and 85 represent another set of sample designer genes that can express a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 2-methyl-1-butanol production pathway numerically labeled as 34, 35, 03-05, 36-39, 53-55, 42 and 56; while SEQ ID NOS: 58-61, 74-84, 69 and 85 represent a set of sample designer genes that can express another Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 2-methyl-1-butanol production pathway of 34, 35, 03, 04, 45-55, 42 and 56 in FIG. 5. These designer genes can be used in combination with other pathway gene(s) to express certain other pathways such as a Calvin-cycle Fructose-6-phosphate branched 2-methyl-1-butanol production pathway numerically labeled as 13-26, 36-39, 53-55, 42 and 56 (and/or, as 13-25, 45-55, 42 and 56) in FIG. 5 as well. The net results of the designer photosynthetic NADPH-enhanced pathways in working with the Calvin cycle are production of 2-methyl-1-butanol [CH₃CH₂CH(CH₃)CH₂OH] from carbon dioxide (CO₂) and water (H₂O) using photosynthetically generated ATP and NADPH according to the following process reaction:



Calvin-Cycle-Channeled Pathways for Production of Isobutanol and 3-Methyl-1-Butanol

[0213] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into isobutanol by using, for example, a set of enzymes consisting of (as shown with numerical labels 34, 35, 03-05, 53-55, 42, 43 (or 44) in FIG. 6): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, 2-keto acid decarboxylase 42, and NAD-dependent alcohol dehydrogenase 43 (or NADPH-dependent alcohol dehydrogenase 44). The net result of this pathway in working with the Calvin cycle is photobiological production of isobutanol ((CH₃)₂CHCH₂OH) from carbon dioxide (CO₂) and water (H₂O) using photosynthetically generated ATP and NADPH according to the following process reaction:



[0214] According to another embodiment, a designer Calvin-cycle-channeled pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into 3-methyl-1-butanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels

34, 35, 03-05, 53-55, 40, 38, 39, 42, 43 (or 44/57) in FIG. 6): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, 2-isopropylmalate synthase 40, 3-isopropylmalate dehydratase 38, 3-isopropylmalate dehydrogenase 39, 2-keto acid decarboxylase 42, and NAD-dependent alcohol dehydrogenase 43 (or NADPH-dependent alcohol dehydrogenase 44; or more preferably, 3-methylbutanal reductase 57). The net result of this pathway in working with the Calvin cycle is photobiological production of 3-methyl-1-butanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) using photosynthetically generated ATP and NADPH according to the following process reaction:



[0215] These designer pathways (FIG. 6) share a number of designer pathway enzymes with those of FIGS. 4 and 5, except that a 3-methylbutanal reductase 57 is preferably used for production of 3-methyl-1-butanol; they all have a common feature of using an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 as an NADPH/NADH conversion mechanism to convert certain amount of photosynthetically generated NADPH to NADH which can be used by NADH-requiring pathway enzymes such as an NADH-requiring alcohol dehydrogenase 43.

[0216] SEQ ID NO: 86 presents example 86 of a designer nirA-promoter-controlled 3-Methylbutanal Reductase (57) DNA construct (1420 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1280) selected/modified from the sequences of a *Saccharomyces cerevisiae* S288c 3-Methylbutanal reductase (DAA10635), a 120-bp rbcS terminator from BP1 (1281-1400), and a PCR RE primer (1401-1420) at the 3' end.

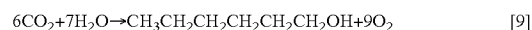
[0217] SEQ ID NOS: 58-62, 82-84, 69, 70 (or 71) represent a set of sample designer genes that can express a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced isobutanol production pathway (34, 35, 03-05, 53-55, 42, 43 or 44); while SEQ ID NOS: 58-62, 82-84, 65-67, 69 and 86 represent another set of sample designer genes that can express a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 3-methyl-1-butanol production pathway (34, 35, 03-05, 53-55, 40, 38, 39, 42, and 57 in FIG. 6).

[0218] These designer genes can be used with certain other designer genes to express certain other pathways such as a Calvin-cycle Fructose-6-phosphate-branched 3-methyl-1-butanol production pathway shown as 13-26, 53-54, 39-40, 42 and 57 (or 43/44) in FIG. 6 as well. The net results of the designer photosynthetic NADPH-enhanced pathways in working with the Calvin cycle are also production of isobutanol ($(\text{CH}_3)_2\text{CHCH}_2\text{OH}$) and/or 3-methyl-1-butanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) using photosynthetically generated ATP and NADPH.

Designer Calvin-Cycle-Channeled Pathways for Production of 1-Hexanol and 1-Octanol

[0219] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that

takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 1-hexanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-10, 07'-12' in FIG. 7): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, pyruvate-ferredoxin oxidoreductase 06, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, designer 3-ketothiolase 07', designer 3-hydroxyacyl-CoA dehydrogenase 08', designer enoyl-CoA dehydratase 09', designer 2-enoyl-CoA reductase 10', designer acyl-CoA reductase 11', and hexanol dehydrogenase 12'. The net result of this designer pathway in working with the Calvin cycle is photobiological production of 1-hexanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) using photosynthetically generated ATP and NADPH according to the following process reaction:



[0220] According to another embodiment, a designer Calvin-cycle-channeled pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into 1-octanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-10, 07'-10', and 07''-12'' in FIG. 7): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, pyruvate-ferredoxin oxidoreductase 06, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, designer 3-ketothiolase 07', designer 3-hydroxyacyl-CoA dehydrogenase 08', designer enoyl-CoA dehydratase 09', designer 2-enoyl-CoA reductase 10', designer 3-ketothiolase 07'', designer 3-hydroxyacyl-CoA dehydrogenase 08'', designer enoyl-CoA dehydratase 09'', designer 2-enoyl-CoA reductase 10'', designer acyl-CoA reductase 11'', and octanol dehydrogenase 12''.

[0221] These pathways represent a significant upgrade in the pathway designs with part of a previously disclosed 1-butanol production pathway (03-10). The key feature is the utilization of an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 as a mechanism for NADPH/NADH conversion to drive an NADH-requiring designer hydrocarbon chain elongation pathway (07'-10') for 1-hexanol production (07'-12' as shown in FIG. 7).

[0222] SEQ ID NOS: 87-92 represent a set of designer genes that can express the designer hydrocarbon chain elongation pathway for 1-hexanol production (07'-12' as shown in FIG. 7). Briefly, SEQ ID NO: 87 presents example 87 of a designer nirA-promoter-controlled 3-Ketothiolase (07') DNA construct (1540 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1400) selected/modified from the sequences of a *Geobacillus kaustophilus* 3-Ketothiolase (YP_147173), a 120-bp rbcS terminator from BP1 (1401-1520), and a PCR RE primer (1521-1540).

[0223] SEQ ID NO: 88 presents example 88 of a designer nirA-promoter-controlled 3-Hydroxyacyl-CoA Dehydrogenase (08') DNA construct (1231 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-en-

coding sequence (252-1091) selected/modified from the sequences of a *Syntrophothermus lipocalidus* 3-Hydroxyacyl-CoA dehydrogenase (YP_003702743), a 120-bp rbcS terminator from BP1 (1092-1211), and a PCR RE primer (1212-1231).

[0224] SEQ ID NO: 89 presents example 89 of a designer nirA-promoter-controlled Enoyl-CoA Dehydratase (09') DNA construct (1162 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1022) selected/modified from the sequences of a *Bordetella petrii* Enoyl-CoA dehydratase (CAP41574), a 120-bp rbcS terminator from BP1 (1023-1442), and a PCR RE primer (1443-1162) at the 3' end.

[0225] SEQ ID NO: 90 presents example 90 of a designer nirA-promoter-controlled 2-Enoyl-CoA Reductase (10') DNA construct (1561 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1421) selected/modified from the sequences of a *Xanthomonas campestris* 2-Enoyl-CoA Reductase (CAP53709), a 120-bp rbcS terminator from BP1 (1422-1541), and a PCR RE primer (1542-1561).

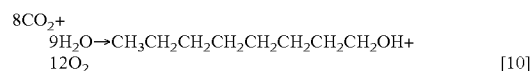
[0226] SEQ ID NO: 91 presents example 91 of a designer nirA-promoter-controlled Acyl-CoA Reductase (11') DNA construct (1747 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1607) selected/modified from the sequences of a *Clostridium cellulovorans* Acyl-CoA reductase (YP_003845606), a 120-bp rbcS terminator from BP1 (1608-1727), and a PCR RE primer (1728-1747).

[0227] SEQ ID NO: 92 presents example 92 of a designer nirA-promoter-controlled Hexanol Dehydrogenase (12') DNA construct (1450 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-1310) selected/modified from the sequences of a *Mycobacterium chubuense* hexanol dehydrogenase (ACZ56328), a 120-bp rbcS terminator from BP1 (1311-1430), and a PCR RE primer (1431-1450).

[0228] SEQ ID NO: 93 presents example 93 of a designer nirA-promoter-controlled Octanol Dehydrogenase (12'') DNA construct (1074 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-934) selected/modified from the sequences of a *Drosophila subobscura* octanol dehydrogenase (ABO65263), a 120-bp rbcS terminator from BP1 (935-1054), and a PCR RE primer (1055-1074) at the 3' end.

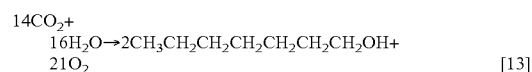
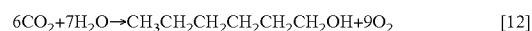
[0229] Note, the designer enzymes of SEQ ID NOS: 87-91 have certain broad substrate specificity. Consequently, they can also be used as designer 3-ketothiolase 07'', designer 3-hydroxyacyl-CoA dehydrogenase 08'', designer enoyl-CoA dehydratase 09'', designer 2-enoyl-CoA reductase 10'', and designer acyl-CoA reductase 11''. Therefore, SEQ ID NOS: 87-91 and 93 represent a set of designer genes that can express another designer hydrocarbon chain elongation pathway for 1-octanol production (07'40' and 07''-12'' as shown in FIG. 7). SEQ ID NO: 93 (encoding for octanol dehydrogenase 12'') is one of the key designer genes that enable production of 1-octanol production in this pathway. The net result of this pathway in working with the Calvin cycle are photobiological production of 1-octanol

(CH₃CH₂CH₂CH₂CH₂CH₂CH₂CH₂OH) from carbon dioxide (CO₂) and water (H₂O) using photosynthetically generated ATP and NADPH according to the following process reaction:



Calvin-Cycle-Channeled Pathways for Production of 1-Pentanol, 1-Hexanol and 1-Heptanol

[0230] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 1-pentanol, 1-hexanol, and/or 1-heptanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-05, 36-41, 39, 39'-43', 39''-43'', 12', and 39'''-43''' in FIG. 8): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, citramalate synthase 36, 2-methylmalate dehydratase 37, 3-isopropylmalate dehydratase 38, 3-isopropylmalate dehydrogenase 39, 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39'', designer 2-keto acid decarboxylase 42', short-chain alcohol dehydrogenase 43', hexanol dehydrogenase 12'', designer isopropylmalate synthase 40'', designer isopropylmalate isomerase 41'', designer 3-isopropylmalate dehydrogenase 39'', designer 2-keto acid decarboxylase 42'', and designer short-chain alcohol dehydrogenase 43''. This designer pathway works with the Calvin cycle using photosynthetically generated ATP and NADPH for photobiological production of 1-pentanol (CH₃CH₂CH₂CH₂CH₂OH), 1-hexanol (CH₃CH₂CH₂CH₂CH₂CH₂OH), and/or 1-heptanol (CH₃CH₂CH₂CH₂CH₂CH₂CH₂OH) from carbon dioxide (CO₂) and water (H₂O) according to the following process reactions:



[0231] According to another embodiment, a designer Calvin-cycle-channeled pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into 1-pentanol, 1-hexanol, and/or 1-heptanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03, 04, 45-52, 40, 41, 39, 39'-43', 39''-43'', 12', and 39'''-43''' in FIG. 8): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, phosphoenolpyruvate carboxylase 45, aspartate aminotransferase 46, aspartokinase 47, aspartate-semialdehyde dehydrogenase 48, homoserine dehydrogenase 49, homoserine kinase 50, threonine synthase 51, threonine ammonia-lyase 52, 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopro-

pylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', short-chain alcohol dehydrogenase 43', hexanol dehydrogenase 12', designer isopropylmalate synthase 40", designer isopropylmalate isomerase 41", designer 3-isopropylmalate dehydrogenase 39", designer 2-keto acid decarboxylase 42", and designer short-chain alcohol dehydrogenase 43".

[0232] These pathways (FIG. 8) share a common feature of using an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 as a mechanism for NADPH/NADH conversion to drive production of 1-pentanol, 1-hexanol, and/or 1-heptanol through a designer Calvin-cycle-channeled pathway in combination with a designer hydrocarbon chain elongation pathway (40', 41', 39'). This embodiment also takes the advantage of the broad substrate specificity (promiscuity) of 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, 2-keto acid decarboxylase 42, and short-chain alcohol dehydrogenase 43 so that they can be used also as: designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', and short-chain alcohol dehydrogenase 43'; isopropylmalate synthase 40", designer isopropylmalate isomerase 41", designer 3-isopropylmalate dehydrogenase 39", designer 2-keto acid decarboxylase 42", and designer short-chain alcohol dehydrogenase 43".

[0233] In this case, proper selection of a short-chain alcohol dehydrogenase with certain promiscuity is also essential. SEQ ID NO: 94 presents example 94 of a designer nirA-promoter-controlled Short Chain Alcohol Dehydrogenase DNA construct (1096 bp) that includes a PCR FD primer (sequence 1-20), a 231-bp nirA promoter from *Thermosynechococcus elongatus* BP1 (21-251), an enzyme-encoding sequence (252-956) selected/modified from the sequences of a *Pyrococcus furiosus* DSM 3638 Short chain alcohol dehydrogenase (AAC25556), a 120-bp rbcS terminator from BP1 (957-1076), and a PCR RE primer (1077-1096) at the 3' end.

[0234] Therefore, SEQ ID NOS: 58-69 and 94 represent a set of designer genes that can express a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway for production of 1-pentanol, 1-hexanol, and/or 1-heptanol as shown with numerical labels 34, 35, 03-05, 36-41, 39, 39'-43', 39"-43" in FIG. 8. Similarly, SEQ ID NOS: 58-61, 74-81, 66-69, and 94 represent another set of sample designer genes that can express another Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway for production of 1-pentanol, 1-hexanol, and/or 1-heptanol as numerically labeled as 34, 35, 03, 04, 45-52, 40, 41, 39, 39'-43', 39"-43" in FIG. 8. Note, both of these two pathways produce alcohol mixtures with different chain lengths rather than single alcohols since all 2-keto acids (such as 2-ketohexanoate, 2-ketaheptanoate, and 2-ketooctanoate) can be converted to alcohol because of the use of the promiscuity of designer 2-keto acid decarboxylase 42' and designer short-chain alcohol dehydrogenase 43'.

[0235] To improve product specificity, it is a preferred practice to use substrate specific designer enzymes. For example, use of substrate specific designer 1-hexanol dehydrogenase 12' (SEQ ID NO: 92) instead of short-chain alcohol dehydrogenase with promiscuity (43') can improve product specificity more toward 1-hexanol. Consequently, SEQ ID NOS: 58-69 and 92 represent a set of designer genes that can

express a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway for production of 1-hexanol as shown with numerical labels 34, 35, 03-05, 36-41, 39, 39'-40', 39'-42' and 12' in FIG. 8.

Designer Calvin-Cycle-Channeled Pathways for Production of 3-Methyl-1-Pentanol, 4-Methyl-1-Hexanol, and 5-Methyl-1-Heptanol

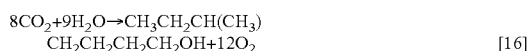
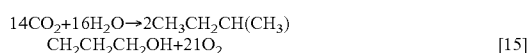
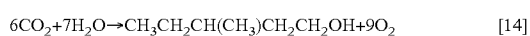
[0236] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 3-methyl-1-pentanol, 4-methyl-1-hexanol, and/or 5-methyl-1-heptanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-05, 36-39, 53-55, 39'-43', 39"-43" in FIG. 9): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, citramalate synthase 36, 2-methylmalate dehydratase 37, 3-isopropylmalate dehydratase 38, 3-isopropylmalate dehydrogenase 39, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', short-chain alcohol dehydrogenase 43', designer isopropylmalate synthase 40", designer isopropylmalate isomerase 41", designer 3-isopropylmalate dehydrogenase 39", designer 2-keto acid decarboxylase 42", and designer short-chain alcohol dehydrogenase 43".

[0237] According to another embodiment, a designer Calvin-cycle-channeled pathway is created that takes the intermediate product, 3-phosphoglycerate, and converts it into 3-methyl-1-pentanol, 4-methyl-1-hexanol, and/or 5-methyl-1-heptanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03, 04, 45-55, 39'-43', 39"-43" in FIG. 9): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, phosphoenolpyruvate carboxylase 45, aspartate aminotransferase 46, aspartokinase 47, aspartate-semialdehyde dehydrogenase 48, homoserine dehydrogenase 49, homoserine kinase 50, threonine synthase 51, threonine ammonia-lyase 52, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', short-chain alcohol dehydrogenase 43', designer isopropylmalate synthase 40", designer isopropylmalate isomerase 41", designer 3-isopropylmalate dehydrogenase 39", designer 2-keto acid decarboxylase 42", and designer short-chain alcohol dehydrogenase 43".

[0238] These pathways (FIG. 9) are similar to those of FIG. 8, except they use acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55 as part of the pathways for production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and/or 5-methyl-1-heptanol. They all share a common feature of using an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 as a mechanism for NADPH/NADH conversion to drive production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and/or 5-methyl-1-heptanol through a designer Calvin-cycle-channeled path-

way in combination with a hydrocarbon chain elongation pathway (40', 41', 39'). This embodiment also takes the advantage of the broad substrate specificity (promiscuity) of 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, 2-keto acid decarboxylase 42, and short-chain alcohol dehydrogenase 43 so that they can also serve as: designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', and short-chain alcohol dehydrogenase 43'; designer isopropylmalate synthase 40'', designer isopropylmalate isomerase 41'', designer 3-isopropylmalate dehydrogenase 39'', designer 2-keto acid decarboxylase 42'', and designer short-chain alcohol dehydrogenase 43''.

[0239] Therefore, SEQ ID NOS: 58-69, 82-84, and 94 represent a set of designer genes that can express a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway for production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and 5-methyl-1-heptanol as shown with numerical labels 34, 35, 03-05, 36-39, 53-55, 39'-43', 39''-43'', and 39'''-43''' in FIG. 9. Similarly, SEQ ID NOS: 58-61, 74-81, 82-84, 66-69, and 94 represent another set of sample designer genes that can express another Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway for production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and/or 5-methyl-1-heptanol as numerically labeled as 34, 35, 03, 04, 45-55, 39'-43', 39''-43'', 39'''-43''' in FIG. 9. The net results of the designer photosynthetic NADPH-enhanced pathways in working with the Calvin cycle are production of 3-methyl-1-pentanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{OH}$), 4-methyl-1-hexanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), and 5-methyl-1-heptanol ($\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) using photosynthetically generated ATP and NADPH according to the following process reactions:



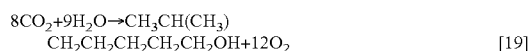
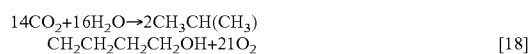
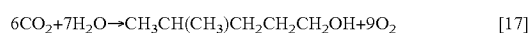
Designer Calvin-Cycle-Channeled Pathways for Production of 4-Methyl-1-Pentanol, 5-Methyl-1-Hexanol, and 6-Methyl-1-Heptanol

[0240] According to one of the various embodiments, a designer Calvin-cycle-channeled pathway is created that takes the Calvin-cycle intermediate product, 3-phosphoglycerate, and converts it into 4-methyl-1-pentanol, 5-methyl-1-hexanol, and 6-methyl-1-heptanol by using, for example, a set of enzymes consisting of (as shown with the numerical labels 34, 35, 03-05, 53-55, 40, 38, 39, 39'-43', 39''-43'', and 39'''-43''' in FIG. 10): NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35, phosphoglycerate mutase 03, enolase 04, pyruvate kinase 05, acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55, isopropylmalate synthase 40, dehydratase 38, 3-isopropylmalate dehydrogenase 39, designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', short-chain alcohol dehydrogenase 43', designer isopropylmalate synthase 40'', designer

isopropylmalate isomerase 41'', designer 3-isopropylmalate dehydrogenase 39'', designer 2-keto acid decarboxylase 42'', and designer short-chain alcohol dehydrogenase 43''.

[0241] This pathway (FIG. 10) is similar to those of FIG. 8, except that it does not use citramalate synthase 36 and 2-methylmalate dehydratase 37, but uses acetolactate synthase 53, ketol-acid reductoisomerase 54, dihydroxy-acid dehydratase 55 as part of the pathways for production of 4-methyl-1-pentanol, 5-methyl-1-hexanol, and/or 6-methyl-1-heptanol. They all share a common feature of using an NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase 34 and an NAD-dependent glyceraldehyde-3-phosphate dehydrogenase 35 as a mechanism for NADPH/NADH conversion to drive production of 3-methyl-1-butanol, 4-methyl-1-butanol, and 5-methyl-1-butanol through a Calvin-cycle-channeled pathway in combination with a designer hydrocarbon chain elongation pathway (40', 41', 39'). This embodiment also takes the advantage of the broad substrate specificity (promiscuity) of 2-isopropylmalate synthase 40, isopropylmalate isomerase 41, 3-isopropylmalate dehydrogenase 39, 2-keto acid decarboxylase 42, and short-chain alcohol dehydrogenase 43 so that they may also serve as: designer isopropylmalate synthase 40', designer isopropylmalate isomerase 41', designer 3-isopropylmalate dehydrogenase 39', designer 2-keto acid decarboxylase 42', and short-chain alcohol dehydrogenase 43', designer isopropylmalate synthase 40'', designer isopropylmalate isomerase 41'', designer 3-isopropylmalate dehydrogenase 39'', designer 2-keto acid decarboxylase 42'', and designer short-chain alcohol dehydrogenase 43''.

[0242] Therefore, SEQ ID NOS: 58-62, 82-84, 65-69 and 94 represent a set of sample designer genes that can be used to express a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway for production of 4-methyl-1-pentanol, 5-methyl-1-hexanol, and/or 6-methyl-1-heptanol as shown with numerical labels 34, 35, 03-05, 53-55, 40, 38, 39, 39'-43', 39''-43'', and 39'''-43''' in FIG. 10. The net results of the designer photosynthetic NADPH-enhanced pathway in working with the Calvin cycle are production of 4-methyl-1-pentanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 5-methyl-1-hexanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), and 6-methyl-1-heptanol ($\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from carbon dioxide (CO_2) and water (H_2O) using photosynthetically generated ATP and NADPH according to the following process reactions:



Designer Oxyphotobacteria with Calvin-Cycle-Channeled Pathways for Production of Butanol and Related Higher Alcohols

[0243] According to one of the various embodiments, use of designer DNA constructs in genetic transform of certain oxyphotobacteria hosts can create various designer transgenic oxyphotobacteria with Calvin-cycle-channeled pathways for photobiological production of butanol and related higher alcohols from carbon dioxide and water. To ensure biosafety for use of the designer transgenic photosynthetic organism-based biofuels production technology, it is a preferred practice to incorporate biosafety-guarded features into

the designer transgenic photosynthetic organisms as well. Therefore, in accordance with the present invention, various designer photosynthetic organisms including designer transgenic oxyphotobacteria are created with a biosafety-guarded photobiological biofuel-production technology based on cell-division-controllable designer transgenic photosynthetic organisms. The cell-division-controllable designer photosynthetic organisms contain two key functions: a designer biosafety mechanism(s) and a designer biofuel-production pathway(s). The designer biosafety feature(s) is conferred by a number of mechanisms including: a) the inducible insertion of designer proton-channels into cytoplasm membrane to permanently disable any cell division and/or mating capability, b) the selective application of designer cell-division-cycle regulatory protein or interference RNA (iRNA) to permanently inhibit the cell division cycle and preferably keep the cell at the G₁ phase or G₀ state, and c) the innovative use of a high-CO₂-requiring host photosynthetic organism for expression of the designer biofuel-production pathway(s). The designer cell-division-control technology can help ensure biosafety in using the designer organisms for biofuel production.

[0244] Oxyphotobacteria (including cyanobacteria and oxychlorobacteria) that can be selected for use as host organisms to create designer transgenic oxyphotobacteria for photobiological production of butanol and related higher alcohols include (but not limited to): *Thermosynechococcus elongatus* BP-1, *Nostoc* sp. PCC 7120, *Synechococcus elongatus* PCC 6301, *Synechococcus* sp. strain PCC 7942, *Synechococcus* sp. strain PCC 7002, *Synechocystis* sp. strain PCC 6803, *Prochlorococcus marinus* MED4, *Prochlorococcus marinus* MIT 9313, *Prochlorococcus marinus* NATL1A, *Prochlorococcus* SS120, *Spirulina platensis* (*Arthrospira platensis*), *Spirulina pacifica*, *Lyngbya majuscula*, *Anabaena* sp., *Synechocystis* sp., *Synechococcus elongatus*, *Synechococcus* (MC-A), *Trichodesmium* sp., *Richelia intracellularis*, *Synechococcus* WH7803, *Synechococcus* WH8102, *Nostoc punctiforme*, *Synechococcus* sp. strain PCC 7943, *Synechocystis* PCC 6714 phycocyanin-deficient mutant PD-1, *Cyanothece* strain 51142, *Cyanothece* sp. CCY0110, *Oscillatoria limosa*, *Lyngbya majuscula*, *Symploca muscorum*, *Gloeobacter violaceus*, *Prochloron didemni*, *Prochlorothrix hollandica*, *Prochlorococcus marinus*, *Prochlorococcus* SS120, *Synechococcus* WH8102, *Lyngbya majuscula*, *Symploca muscorum*, *Synechococcus bigranulatus*, cryophilic *Oscillatoria* sp., *Phormidium* sp., *Nostoc* sp.-1, *Calothrix parietina*, thermophilic *Synechococcus bigranulatus*, *Synechococcus lividus*, thermophilic *Mastigocladus laminosus*, *Chlorogloeopsis fritschii* PCC 6912, *Synechococcus vulcanus*, *Synechococcus* sp. strain MA4, *Synechococcus* sp. strain MA19, and *Thermosynechococcus elongatus*.

[0245] According to one of the examples, use of designer DNA constructs such as SEQ ID NOS: 58-94 in genetic transform of certain oxyphotobacteria hosts such as *Thermosynechococcus elongatus* BP1 can create a series of designer transgenic oxyphotobacteria with Calvin-cycle-channeled pathways for production of butanol and related higher alcohols. Consequently, SEQ ID NOS: 58-61, 74-81, 66-69, and 72 (and/or 73) represent a designer transgenic oxyphotobacterium such as a designer transgenic *Thermosynechococcus* that comprises the designer genes of a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (numerically labeled as 34, 35, 03, 04, 45-52, 39-42, and 12 in FIG. 4) for photobiological production of 1-butanol

from carbon dioxide and water. SEQ ID NOS: 58-69 and 72 (and/or 73) represent another designer transgenic oxyphotobacterium such as designer transgenic *Thermosynechococcus* that comprises the designer genes of a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (numerically labeled as 34, 35, 03-05, 36-42, and 12 in FIG. 4) for photobiological production of 1-butanol from carbon dioxide and water as well.

[0246] Similarly, SEQ ID NOS: 58-66, 82-84, 69 and 85 represent another designer transgenic oxyphotobacterium such as designer transgenic *Thermosynechococcus* with a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (numerically labeled as 34, 35, 03-05, 36-39, 53-55, 42 and 56 in FIG. 5) for photobiological production of 2-methyl-1-butanol production from carbon dioxide and water; while SEQ ID NOS: 58-61, 74-84, 69 and 85 represent another designer transgenic *Thermosynechococcus* with a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 2-methyl-1-butanol production pathway (34, 35, 03, 04, 45-55, 42 and 56 in FIG. 5) for photobiological production of 2-methyl-1-butanol production from carbon dioxide and water.

[0247] SEQ ID NOS: 58-63, 82-84, 69, 70 (or 71) represent another designer transgenic oxyphotobacterium such as designer transgenic *Thermosynechococcus* with a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced isobutanol production pathway (34, 35, 03-05, 53-5, 42, 43 or 44); while SEQ ID NOS: 58-62, 81-83, 65-67, 69 and 86 represent another designer transgenic *Thermosynechococcus* with a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced 3-methyl-1-butanol production pathway (numerical labels 34, 35, 03-05, 53-55, 40, 38, 39, 42, and 57 in FIG. 6).

[0248] SEQ ID NOS: 87-92 represent another designer transgenic *Thermosynechococcus* with a designer hydrocarbon chain elongation pathway (07'-12' as shown in FIG. 7) for photobiological production of 1-hexanol. SEQ ID NOS: 87-91 and 93 represent another designer transgenic *Thermosynechococcus* with a designer hydrocarbon chain elongation pathway (07'-10' and 07"-12" as shown in FIG. 7) for photobiological production of 1-octanol.

[0249] SEQ ID NOS: 58-69 and 92 represent another designer transgenic *Thermosynechococcus* with a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (34, 35, 03-05, 36-41, 39, 39'-40', 39'-42' and 12' in FIG. 8) for photobiological production of 1-hexanol from carbon dioxide and water.

[0250] SEQ ID NOS: 58-69, 82-84, and 94 represent a designer transgenic *Thermosynechococcus* with a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (34, 35, 03-05, 36-39, 53-55, 39'-43', 39'-43', 39"-43" in FIG. 9) for production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and 5-methyl-1-heptanol from carbon dioxide and water. Similarly, SEQ ID NOS: 58-61, 74-81, 82-84, 66-69, and 94 represent another designer transgenic *Thermosynechococcus* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (34, 35, 03, 04, 45-55, 39'-43', 39'-43', 39"-43" in FIG. 9) for photobiological production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and 5-methyl-1-heptanol from carbon dioxide and water as well.

[0251] SEQ ID NOS: 58-62, 82-84, 65-69 and 94 represent a designer transgenic *Thermosynechococcus* with a designer Calvin-cycle 3-phosphoglycerate-branched photosynthetic

NADPH-enhanced pathway labels (34, 35, 03-05, 53-55, 40, 38, 39, 39'-43', 39''-43'', and 39'''-43''') in FIG. 10) for photobiological production of 4-methyl-1-pentanol, 5-methyl-1-hexanol, and/or 6-methyl-1-heptanol from carbon dioxide and water.

[0252] Use of other host oxyphotobacteria such as *Synechococcus* sp. strain PCC 7942, *Synechocystis* sp. strain PCC 6803, *Prochlorococcus marinus*, *Cyanothece* sp. ATCC 51142, for genetic transformation with proper designer DNA constructs (genes) can create other designer oxyphotobacteria for photobiological production of butanol and higher alcohols as well. For example, use of *Synechococcus* sp. strain PCC 7942 as a host organism in genetic transformation with SEQ ID NOS: 95-98 (and/or 99) can create a designer transgenic *Synechococcus* for photobiological production of 1-butanol. Briefly, SEQ ID NO: 95 presents example 95 of a detailed DNA construct (1438 base pairs (bp)) of a designer NADPH-dependent Glyceraldehyde-3-Phosphate-Dehydrogenase (34) gene that includes a PCR FD primer (sequence by 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* sp. strain PCC 7942 (freshwater cyanobacterium) nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1110) selected and modified from a *Staphylococcus* NADPH-dependent glyceraldehyde-3-phosphate-dehydrogenase sequence (GenBank accession number: YP_003471459), a 308-bp *Synechococcus* *rbcS* terminator (1111-1418), and a PCR RE primer (1419-1438).

[0253] SEQ ID NO: 96 presents example 96 of a detailed DNA construct (1447 bp) of a designer NAD-dependent Glyceraldehyde-3-Phosphate-Dehydrogenase (35) gene that includes a PCR FD primer (sequence by 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1119) selected from a *Staphylococcus aureus* NAD-dependent glyceraldehyde-3-phosphate-dehydrogenase sequence (GenBank accession number: ADC36961), a 308-bp *Synechococcus* *rbcS* terminator (1120-1427), and a PCR RE primer (1428-1447).

[0254] SEQ ID NO: 97 presents example 97 of a detailed DNA construct (2080 bp) of a designer 2-Keto Acid Decarboxylase (42) gene that includes a PCR FD primer (sequence by 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1752) selected from a *Lactococcus lactis* branched-chain alpha-ketoacid decarboxylase (GenBank accession number: AAS49166), a 308-bp *Synechococcus* *rbcS* terminator (1753-2060), and a PCR RE primer (2061-2080).

[0255] SEQ ID NO: 98 presents a detailed DNA construct (1603 bp) of a designer NADH-dependent butanol dehydrogenase (12a) gene that include a PCR FD primer (sequence by 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1275) selected from a *Clostridium* NADH-dependent butanol dehydrogenase (GenBank accession number: ADO12118), a 308-bp *Synechococcus* *rbcS* terminator (1276-1583), and a PCR RE primer (1584-1603).

[0256] SEQ ID NO: 99 presents example 99 of a detailed DNA construct (1654 bp) of a designer NADPH-dependent Butanol Dehydrogenase (12b) gene including: a PCR FD primer (sequence by 1-20), a 88-bp *nirA* promoter (21-108) selected from the *Synechococcus* nitrite-reductase-gene promoter sequence, an enzyme-encoding sequence (109-1326)

selected from a *Butyrivibrio* NADPH-dependent butanol dehydrogenase (GenBank: EFF67629), a 308-bp *Synechococcus* *rbcS* terminator (1327-1634), and a PCR RE primer (1635-1654).

[0257] Note, in the designer transgenic *Synechococcus* that is represented by SEQ ID NOS: 95-98 (and/or 99), *Synechococcus*'s native enzymes of 03-05, 36-41 and 45-52 are used in combination with the designer *nirA*-promoter-controlled enzymes of 34, 35, 42 and 12 [encoded by SEQ ID NOS: 95-98 (and/or 99)] to confer the Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathways for photobiological production of 1-butanol from carbon dioxide and water (FIG. 4).

[0258] Similarly, use of *Synechocystis* sp. strain PCC 6803 as a host organism in genetic transformation with SEQ ID NOS: 100-102 (and/or 103) creates a designer transgenic *Synechocystis* for photobiological production of 1-butanol. Briefly, SEQ ID NO: 100 presents example 100 of a designer *nirA*-promoter-controlled NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase (35) DNA construct (1440 bp) that includes a PCR FD primer (sequence 1-20), a 89-bp *Synechocystis* sp. strain PCC 6803 nitrite-reductase *nirA* promoter (21-109), an enzyme-encoding sequence (110-1011) selected from a *Streptococcus pyogenes* NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase (GenBank: YP_002285269), a 409-bp *Synechocystis* sp. PCC 6803 *rbcS* terminator (1012-1420), and a PCR RE primer (1421-1440).

[0259] SEQ ID NO: 101 presents example 101 of a designer *nirA*-promoter-controlled 2-Keto Acid Decarboxylase (42) DNA construct (2182 bp) that includes a PCR FD primer (sequence 1-20), a 89-bp *Synechocystis* sp. strain PCC 6803 nitrite-reductase *nirA* promoter (21-109), an enzyme-encoding sequence (110-1753) selected from a *Lactococcus lactis* branched-chain alpha-ketoacid decarboxylase (GenBank: AAS49166), a 409-bp *Synechocystis* sp. PCC 6803 *rbcS* terminator (1754-2162), and a PCR RE primer (2163-2182).

[0260] SEQ ID NO: 102 presents example 102 of a designer *nirA*-promoter-controlled NADH-dependent Butanol Dehydrogenase (12a) DNA construct (1705 bp) that includes a PCR FD primer (sequence 1-20), a 89-bp *Synechocystis* sp. strain PCC 6803 nitrite-reductase *nirA* promoter (21-109), an enzyme-encoding sequence (110-1276) selected from a *Clostridium carboxidivorans* P7 NADH-dependent butanol dehydrogenase (GenBank: ADO12118), a 409-bp *Synechocystis* sp. PCC 6803 *rbcS* terminator (1277-1685), and a PCR RE primer (1686-1705).

[0261] SEQ ID NO: 103 presents example 103 of a designer *nirA*-promoter-controlled NADPH-dependent butanol dehydrogenase (12b) DNA construct (1756 bp) that includes a PCR FD primer (sequence 1-20), a 89-bp *Synechocystis* sp. strain PCC 6803 nitrite-reductase *nirA* promoter (21-109), an enzyme-encoding sequence (110-1327) selected from a *Butyrivibrio crossotus* NADPH-dependent butanol dehydrogenase (GenBank: EFF67629), a 409-bp *Synechocystis* sp. PCC 6803 *rbcS* terminator (1328-1736), and a PCR RE primer (1737-1756).

[0262] Note, in the designer transgenic *Synechocystis* that contains the designer genes of SEQ ID NOS: 100-102 (and/or 103), *Synechocystis*'s native enzymes of 34, 03-05, 36-41 and 45-52 are used in conjunction with the designer *nirA*-promoter-controlled enzymes of 35, 42 and 12 [encoded by SEQ ID NOS: 100-102 (and/or 103)] to confer the Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-en-

hanced pathways for photobiological production of 1-butanol from carbon dioxide and water (FIG. 4).

[0263] Use of *Nostoc* sp. strain PCC 7120 as a host organism in genetic transformation with SEQ ID NOS: 104-109 can create a designer transgenic *Nostoc* for photobiological production of 2-methyl-1-butanol (FIG. 5). Briefly, SEQ ID NO: 104 presents example 104 of a designer hox-promoter-controlled NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase (35) DNA construct (1655 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1203) selected/modified from the sequence of a *Streptococcus pyogenes* NZ131 NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (GenBank: YP_002285269), a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1204-1635), and a PCR RE primer (1636-1655).

[0264] SEQ ID NO: 105 presents example 105 of a designer hox-promoter-controlled Acetolactate Synthase (53) DNA construct (2303 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1851) selected/modified from the sequence of a *Thermosynechococcus elongatus* BP-1 acetolactate synthase (GenBank: NP_682614), a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1852-2283), and a PCR RE primer (2284-2303).

[0265] SEQ ID NO: 106 presents example 106 of a designer hox-promoter-controlled Ketol-Acid Reductoisomerase (54) DNA construct (1661 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1209) selected/modified from the sequence of a *Calditerrivibrio nitroreducens* ketol-acid reductoisomerase (GenBank: YP_004050904), a 432-bp *Nostoc* sp. gor terminator (1210-1641), and a PCR RE primer (1642-1661).

[0266] SEQ ID NO: 107 presents example 107 of a designer hox-promoter-controlled Dihydroxy-Acid Dehydratase (55) DNA construct (2324 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1872) selected/modified from the sequence of a *Marivirga tractuosa* DSM 4126 dihydroxy-acid dehydratase (GenBank: YP_004053736), a 432-bp *Nostoc* sp. gor terminator (1873-2304), and a PCR RE primer (2305-2324).

[0267] SEQ ID NO: 108 presents example 108 of a designer hox-promoter-controlled branched-chain alpha-Ketoacid Decarboxylase (42) DNA construct (2288 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1836) selected/modified from the sequence of a *Lactococcus lactis* branched-chain alpha-ketoacid decarboxylase (GenBank: AAS49166), a 432-bp *Nostoc* sp. gor terminator (1837-2268), and a PCR RE primer (2269-2288).

[0268] SEQ ID NO: 109 presents example 109 of a designer hox-promoter-controlled 2-Methylbutyraldehyde Reductase (56) DNA construct (1613 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1461) selected/modified from the sequence of a *Schizosaccharomyces japonicus* y 2-methylbutyraldehyde reductase

(GenBank: XP_002173231), a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1462-1893), and a PCR RE primer (1894-1613).

[0269] Note, in the designer transgenic *Nostoc* that contains designer hox-promoter-controlled genes of SEQ ID NOS: 104-109, *Nostoc*'s native enzymes (genes) of 34, 03-05, 36-39 and 45-52 are used in combination with the designer hox-promoter-controlled enzymes of 35, 53-55, 42 and 56 (encoded by DNA constructs of SEQ ID NOS: 104-109) to confer the Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathways for photobiological production of 2-methyl-1-butanol from carbon dioxide and water (FIG. 5).

[0270] Use of *Prochlorococcus marinus* MIT 9313 as a host organism in genetic transformation with SEQ ID NOS: 110-122 can create a designer transgenic *Prochlorococcus marinus* for photobiological production of isobutanol and/or 3-methyl-1-butanol (FIG. 6). Briefly, SEQ ID NO:110 presents example 110 for a designer groE-promoter-controlled NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase (35) DNA construct (1300 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT 9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1159) selected from a *Vibrio cholerae* MJ-1236 NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase (GenBank: ACQ61431), a 121-bp *Prochlorococcus marinus* MIT9313 rbcS terminator (1160-1280), and a PCR RE primer (1281-1300).

[0271] SEQ ID NO:111 presents example 111 for a designer groE-promoter-controlled Phosphoglycerate Mutase (03) DNA construct (1498 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1357) selected from a *Pelotomaculum thermopropionicum* SI phosphoglycerate mutase (GenBank: YP_001212148), a 121-bp *Prochlorococcus marinus* rbcS terminator (1358-1478), and a PCR RE primer (1479-1498).

[0272] SEQ ID NO:112 presents example 112 for a designer groE-promoter-controlled Enolase (04) DNA construct (1588 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus* heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1447) selected from a *Thermotoga* enolase (GenBank: ABQ46079), a 121-bp *Prochlorococcus marinus* rbcS terminator (1448-1568), and a PCR RE primer (1569-1588).

[0273] SEQ ID NO:113 presents example 113 for a designer groE-promoter-controlled Pyruvate Kinase (05) DNA construct (1717 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1576) selected from a *Thermotoga lettingae* TMO pyruvate kinase (GenBank: YP_001471580), a 121-bp *Prochlorococcus marinus* MIT9313 rbcS terminator (1577-1697), and a PCR RE primer (1698-1717).

[0274] SEQ ID NO:114 presents example 114 for a designer groE-promoter-controlled Acetolactate Synthase (53) DNA construct (2017 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT 9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1876) selected from a *Bacillus licheniformis* ATCC 14580 acetolactate synthase

(GenBank: AAU42663), a 121-bp *Prochlorococcus marinus* MIT 9313 rbcS terminator (1877-1997), and a PCR RE primer (1998-2017).

[0275] SEQ ID NO:115 presents example 115 for a designer groE-promoter-controlled Ketol-Acid Reductoisomerase (54) DNA construct (1588 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1168) selected from a *Thermotoga petrophila* RKU-1 ketol-acid reductoisomerase (GenBank: ABQ46398), a 400-bp *Prochlorococcus marinus* MIT9313 rbcS terminator (1169-1568), and a PCR RE primer (1569-1588).

[0276] SEQ ID NO:116 presents example 116 for a designer groE-promoter-controlled Dihydroxy-Acid Dehydratase (55) DNA construct (1960 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1819) selected from a *Syntrophothermus lipocalidus* DSM 12680 dihydroxy-acid dehydratase (GenBank: ADI02905), a 121-bp *Prochlorococcus marinus* rbcS terminator (1820-1940), and a PCR RE primer (1941-1960).

[0277] SEQ ID NO:117 presents example 117 for a designer groE-promoter-controlled 2-Keto Acid Decarboxylase (42) DNA construct (1945 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus* heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1804) selected from a *Lactococcus lactis* Alpha-ketoisovalerate decarboxylase (GenBank: ADA65057), a 121-bp *Prochlorococcus* rbcS terminator (1805-1925), and a PCR RE primer (1926-1945).

[0278] SEQ ID NO:118 presents example 118 for a designer nirA-promoter-controlled Alcohol Dehydrogenase (43/44) DNA construct (1138 bp) that includes a PCR FD primer (sequence 1-20), a 251-bp *Prochlorococcus* nirA promoter (21-271), an enzyme-encoding sequence (272-997) selected from a *Geobacillus* short chain alcohol dehydrogenase (GenBank: YP_146837), a 121-bp *Prochlorococcus* rbcS terminator (998-1118), and a PCR RE primer (1119-1138).

[0279] Note, in the designer transgenic *Prochlorococcus* that contains the designer genes of SEQ ID NOS: 110-118, *Prochlorococcus*'s native gene (enzyme) of 34 is used in combination with the designer groE and nirA-promoters-controlled genes (enzymes) of 35, 03-05, 53-55, 42 and 43/44 (encoded by DNA constructs of SEQ ID NOS: 110-118) to confer the Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathways for photobiological production of isobutanol from carbon dioxide and water (FIG. 6). Addition of the following four designer groE promoter-controlled genes (SEQ ID NO:119-122) results in another designer transgenic *Prochlorococcus* that can produce both isobutanol and 3-methyl-1-butanol from carbon dioxide and water (35, 03-05, 53-55, 42, 43/44, plus 38-40 and 57 as shown in FIG. 6).

[0280] Briefly, SEQ ID NO:119 presents example 119 for a designer groE-promoter-controlled 2-Isopropylmalate Synthase (40) DNA construct (1816 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1675) selected from a *Pelotomaculum thermopropionicum* S12-isopropylmalate

synthase (GenBank: YP_001211081), a 121-bp *Prochlorococcus marinus* rbcS terminator (1676-1796), and a PCR RE primer (1797-1816).

[0281] SEQ ID NO:120 presents example 120 for a designer groE-promoter-controlled 3-Isopropylmalate Dehydratase (38) DNA construct (2199 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), a 3-isopropylmalate dehydratase large subunit-encoding sequence (158-1420) selected from a *Pelotomaculum thermopropionicum* S13-isopropylmalate dehydratase large subunit (GenBank: YP_001211082), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (1421-1557), a 3-isopropylmalate dehydratase small subunit-encoding sequence (1558-2058) selected from a *Pelotomaculum thermopropionicum* S13-isopropylmalate dehydratase small subunit (GenBank: YP_001211083), a 121-bp *Prochlorococcus marinus* rbcS terminator (2059-2179), and a PCR RE primer (2180-2199).

[0282] SEQ ID NO:121 presents example 121 for a designer groE-promoter-controlled 3-Isopropylmalate Dehydrogenase (39) DNA construct (1378 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1237) selected from a *Syntrophothermus lipocalidus* DSM 12680 3-isopropylmalate dehydrogenase (GenBank: ADI02898), a 121-bp *Prochlorococcus marinus* rbcS terminator (1238-1358), and a PCR RE primer (1359-1378).

[0283] SEQ ID NO:122 presents example 122 for a designer groE-promoter-controlled 3-Methylbutanal Reductase (57) DNA construct (1327 bp) that includes a PCR FD primer (sequence 1-20), a 137-bp *Prochlorococcus marinus* MIT9313 heat- and light-responsive groE promoter (21-157), an enzyme-encoding sequence (158-1186) selected from a *Saccharomyces cerevisiae* S288c 3-Methylbutanal reductase (GenBank: DAA10635), a 121-bp *Prochlorococcus marinus* MIT9313 rbcS terminator (1187-1307), and a PCR RE primer (1308-1327).

[0284] Note, the use of SEQ ID NOS: 110-117 and 119-122 in genetic transformation of *Prochlorococcus marinus* MIT 9313 creates another designer transgenic *Prochlorococcus marinus* with a groE promoter-controlled designer Calvin-cycle-channeled pathway (identified as 34 (native), 35, 03-05, 53-55, 38-40, 42 and 57 in FIG. 6) for photobiological production of 3-methyl-1-butanol from carbon dioxide and water.

[0285] Use of *Cyanothece* sp. ATCC 51142 as a host organism in genetic transformation with SEQ ID NOS: 123-128 can create a designer transgenic *Cyanothece* for photobiological production of 1-pentanol, 1-hexanol, and/or 1-heptanol (FIG. 8). Briefly, SEQ ID NO:123 presents example 123 for a designer nirA-promoter-controlled 2-Isopropylmalate Synthase (40) DNA construct (2004 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp *Cyanothece* sp. nirA promoter (21-223), an enzyme-encoding sequence (224-1783) selected from a *Hydrogenobacter thermophilus* 2-isopropylmalate synthase sequence (GenBank: BAI69273), a 201-bp *Cyanothece* sp. rbcS terminator (1784-1984), and a PCR RE primer (1985-2004).

[0286] SEQ ID NO:124 presents example 124 for a designer nirA-promoter-controlled Isopropylmalate Isomerase (41) large/small subunits DNA construct (2648 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp

Cyanotheca sp. ATCC 51142 nirA promoter (21-223), an enzyme-large-subunit-encoding sequence (224-1639) selected from a *Anoxybacillus flavithermus* WK1 isopropylmalate isomerase large subunit sequence (GenBank: YP_002314962), a 203-bp *Cyanotheca* sp. ATCC 51142 nirA promoter (1640-1842), an enzyme-small-subunit-encoding sequence (1843-2427) selected from a *Anoxybacillus flavithermus* WK1 isopropylmalate isomerase small subunit sequence (GenBank: YP_002314963), a 201-bp *Cyanotheca* sp. ATCC 51142 rbcS terminator (2428-1628), and a PCR RE primer (2629-2648).

[0287] SEQ ID NO:125 presents example 125 for a designer g nirA-promoter-controlled 3-Isopropylmalate Dehydrogenase (39) DNA construct (1530 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp *Cyanotheca* sp. ATCC 51142 nirA promoter (21-223), an enzyme-encoding sequence (224-1309) selected from a *Thermosynechococcus elongatus* BP-1 3-isopropylmalate dehydrogenase sequence (GenBank: BAC09152), a 201-bp *Cyanotheca* sp. ATCC 51142 rbcS terminator (1310-1310), and a PCR RE primer (1311-1530).

[0288] SEQ ID NO:126 presents example 126 for a designer nirA-promoter-controlled 2-Keto Acid Decarboxylase (42') DNA construct (2088 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp *Cyanotheca* nirA promoter (21-223), an enzyme-encoding sequence (224-1867) selected from a *Lactococcus lactis* 2-keto acid decarboxylase (GenBank: AAS49166), a 201-bp *Cyanotheca* rbcS terminator (1868-2068), and a PCR RE primer (2069-2088).

[0289] SEQ ID NO:127 presents example 127 for a designer nirA-promoter-controlled Hexanol Dehydrogenase (12') DNA construct (1503 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp *Cyanotheca* nirA promoter (21-223), an enzyme-encoding sequence (224-1282) selected from a *Mycobacterium chubuense* hexanol dehydrogenase (GenBank: ACZ56328), a 201-bp *Cyanotheca* rbcS terminator (1283-1483), and a PCR RE primer (1484-1503).

[0290] SEQ ID NO:128 presents example 128 for a designer nirA-promoter-controlled short-chain Alcohol Dehydrogenase (43', 43'') DNA construct (1149 bp) that includes a PCR FD primer (sequence 1-20), a 203-bp *Cyanotheca* sp. ATCC 51142 nirA promoter (21-223), an enzyme-encoding sequence (224-928) selected from a *Pyrococcus furiosus* DSM 3638 Short chain alcohol dehydrogenase (GenBank: AAC25556), a 201-bp *Cyanotheca* sp. ATCC 51142 rbcS terminator (929-1129), and a PCR RE primer (1130-1149).

[0291] Note, in the designer transgenic *Cyanotheca* that contains designer nirA promoter-controlled genes of SEQ ID NOS: 123-127, *Cyanotheca*'s native enzymes of 34,03-05, 36-38, and 45-52 are used in combination with the designer nirA-promoters-controlled enzymes of 35, 39-41 (39'-41', 39'-41'), 42' and 12' (encoded by DNA constructs of SEQ ID NOS: 123-127) to confer the Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathways for photobiological production of 1-hexanol from carbon dioxide and water (FIG. 8). Addition of a designer nirA-promoters-controlled gene (SEQ ID NO: 128) of a short chain alcohol dehydrogenase 43' (43'') with promiscuity results in another designer transgenic *Cyanotheca* containing a Calvin-cycle-channeled pathway (35, 39-41, 39'-43', 39'-43', and 39''-43'' as shown in FIG. 8) that can produce 1-pentanol, 1-hexanol, and 1-hexanol from carbon dioxide and water.

Designer Advanced Photosynthetic Organisms with Calvin-Cycle-Channeled Pathways for Production of Butanol and Related Higher Alcohols

[0292] According to one of the various embodiments, use of certain designer DNA constructs in genetic transformation of eukaryotic photosynthetic organisms such as plant cells, eukaryotic aquatic plants (including, for example, eukaryotic algae, submersed aquatic herbs, duckweeds, water cabbage, water lily, water hyacinth, *Bolbitis heudelotii*, *Cabomba* sp., and seagrasses) can create designer transgenic eukaryotic photosynthetic organisms for production of butanol and related higher alcohols from carbon dioxide and water. Eukaryotic algae that can be selected for use as host organisms to create designer algae for photobiological production of butanol and related higher alcohols include (but not limited to): *Dunaliella salina*, *Dunaliella viridis*, *Dunaliella bardowil*, *Cryptocodinium cohnii*, *Schizochytrium* sp., *Chlamydomonas reinhardtii*, *Platymonas subcordiformis*, *Chlorella fusca*, *Chlorella sorokiniana*, *Chlorella vulgaris*, 'Chlorella' ellipsoidea, *Chlorella* spp., *Haematococcus pluvialis*; *Parachlorella kessleri*, *Betaphycus gelatinum*, *Chondrus crispus*, *Cyanidioschyzon merolae*, *Cyanidium caldarium*, *Galdieria sulphuraria*, *Gelidiella acerosa*, *Gracilaria changii*, *Kappaphycus alvarezii*, *Porphyra miniata*, *Ostreococcus tauri*, *Porphyra yezoensis*, *Porphyridium* sp., *Palmaria palmata*, *Gracilaria* spp., *Isochrysis galbana*, *Kappaphycus* spp., *Laminaria japonica*, *Laminaria* spp., *Monostroma* spp., *Nannochloropsis oculata*, *Porphyra* spp., *Porphyridium* spp., *Undaria pinnatifida*, *Ulva lactuca*, *Ulva* spp., *Undaria* spp., *Phaeodactylum Tricornutum*, *Navicula saprophila*, *Cylindrotheca fusiformis*, *Cyclotella cryptica*, *Euglena gracilis*, *Amphidinium* sp., *Symbiodinium microadriaticum*, *Macrocyctis pyrifera*, *Ankistrodesmus braunii*, *Scenedesmus obliquus*, *Stichococcus* sp., *Platymonas* sp., *Dunaliella sauna*, and *Stephanoptera gracilis*.

[0293] According to another embodiment, the transgenic photosynthetic organism comprises a designer transgenic plant or plant cells selected from the group consisting of aquatic plants, plant cells, green algae, red algae, brown algae, blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria), diatoms, marine algae, freshwater algae, salt-tolerant algal strains, cold-tolerant algal strains, heat-tolerant algal strains, antenna-pigment-deficient mutants, butanol-tolerant algal strains, higher-alcohols-tolerant algal strains, butanol-tolerant oxyphotobacteria, higher-alcohols-tolerant oxyphotobacteria, and combinations thereof.

[0294] According to another embodiment, said transgenic photosynthetic organism comprises a biosafety-guarded feature selected from the group consisting of: a designer proton-channel gene inducible under pre-determined inducing conditions, a designer cell-division-cycle iRNA gene inducible under pre-determined inducing conditions, a high-CO₂-requiring mutant as a host organism for transformation with designer biofuel-production-pathway genes in creating designer cell-division-controllable photosynthetic organisms, and combinations thereof.

[0295] The greater complexity and compartmentalization of eukaryotic plant cells allow for creation of a wider range of photobiologically active designer organisms and novel metabolic pathways compartmentally segregated for production of butanol and/or higher alcohols from water and carbon dioxide. In a eukaryotic algal cell, for example, the translation of designer nuclear genes occurs in cytosol whereas the pho-

tosynthesis/Calvin cycle is located inside an algal chloroplast. This clear separation of algal chloroplast photosynthesis from other subcellular functions such as the functions of cytoplasm membrane, cytosol and mitochondria can be used as an advantage in creation of a biosafety-guarded designer algae through an inducible insertion of designer proton-channels into cytoplasm membrane to permanently disable any cell division and/or mating capability while keeping the algal chloroplast functional work with the designer biofuel production, pathways to produce butanol and related higher alcohols. However, it is essential to genetically deliver designer enzyme(s) into the chloroplast to tame the Calvin cycle and funnel metabolism toward butanol directly from CO₂ and H₂O. This requires more complicated gene design to achieve desirable results.

[0296] According to one of various embodiments, designer Calvin-cycle-channeled pathway enzymes encoded with designer unclear genes are targetedly expressed into algal chloroplast through use of a transit signal peptide sequence. The said signal peptide is selected from the group consisting of the hydrogenase transit-peptide sequences (HydA1 and HydA2), ferredoxin transit-peptide sequence (Frx1), thioredoxin-m transit-peptide sequence (Trx2), glutamine synthase transit-peptide sequence (Gs2), LhcII transit-peptide sequences, PSII-T transit-peptide sequence (PsbT), PSII-S transit-peptide sequence (PsbS), PSII-W transit-peptide sequence (PsbW), CF₀CF₁ subunit-γ transit-peptide sequence (AtpC), CF₀CF₁ subunit-δ transit-peptide sequence (AtpD), CF₀CF₁ subunit-II transit-peptide sequence (AtpG), photosystem I (PSI) transit-peptide sequences, Rubisco SSU transit-peptide sequences, and combinations thereof. Preferred transit peptide sequences include the Hyd1 transit peptide, the Frx1 transit peptide, and the Rubisco SSU transit peptides (such as RbcS2).

[0297] SEQ ID NOS. 129-165 present examples for designer DNA constructs of designer chloroplast-targeted enzymes for creation of designer eukaryotic photosynthetic organisms such as designer algae with Calvin-cycle-channeled photosynthetic NADPH-enhanced pathways for photobiological production of butanol and related higher alcohols. Briefly, SEQ ID NO. 129 presents example 129 for a designer Nia1-promoter-controlled chloroplast-targeted Phosphoglycerate Mutase (03) DNA construct (1910 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a Phosphoglycerate Mutase-encoding sequence (324-1667) selected from *Nostoc azollae* Phosphoglycerate Mutase (ADI65627), a 223-bp *Chlamydomonas* RbcS2 terminator (1668-1890), and a PCR RE primer (1891-1910).

[0298] SEQ ID NO. 130 presents example 130 for a designer Nia1-promoter-controlled chloroplast-targeted Enolase (04) DNA construct (1856 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an Enolase-encoding sequence (324-1613) selected/modified from *Nostoc azollae* Enolase (ADI63801), a 223-bp *Chlamydomonas* RbcS2 terminator (1614-1836), and a PCR RE primer (18837-1856).

[0299] SEQ ID NO. 131 presents example 131 for a designer Nia1-promoter-controlled chloroplast-targeted Pyruvate-Kinase (05) DNA construct (1985 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomo-*

nas reinhardtii Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1742) selected/modified from *Cyanothece* sp. PCC 8802 pyruvate-kinase (YP_003138017), a 223-bp *Chlamydomonas* RbcS2 terminator (1743-1965), and a PCR RE primer (1966-1985).

[0300] SEQ ID NO. 132 presents example 132 for a designer Nia1-promoter-controlled chloroplast-targeted NADPH-dependent Glyceraldehyde-3-Phosphate Dehydrogenase (34) DNA construct (1568 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a NADPH-dependent Glyceraldehyde-3-phosphate dehydrogenase-encoding sequence (324-1325) selected/modified from *Staphylococcus lugdunensis* NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase (ADC87332), a 223-bp *Chlamydomonas* RbcS2 terminator (1326-1548), and a PCR RE primer (1549-1568).

[0301] SEQ ID NO. 133 presents example 133 for a designer Nia1-promoter-controlled chloroplast-targeted NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase (35) DNA construct (1571 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase-encoding sequence (324-1328) selected/modified from *Flavobacteriaceae bacterium* NAD-dependent Glyceraldehyde-3-phosphate dehydrogenase (YP_003095198), a 223-bp *Chlamydomonas* RbcS2 terminator (1329-1551), and a PCR RE primer (1552-1571).

[0302] SEQ ID NO. 134 presents example 134 for a designer Nia1-promoter-controlled chloroplast-targeted Citramalate Synthase (36) DNA construct (2150 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a Citramalate Synthase-encoding sequence (324-1907) selected from *Hydrogenobacter* Citramalate Synthase (ADO45737), a 223-bp *Chlamydomonas* RbcS2 terminator (1908-2130), and a PCR RE primer (2131-2150).

[0303] SEQ ID NO. 135 presents example 135 for a designer Nia1-promoter-controlled chloroplast-targeted 3-Isopropylmalate/(R)-2-Methylmalate Dehydratase (37) large/small subunits DNA construct (3125 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 3-isopropylmalate/(R)-2-methylmalate dehydratase large subunit-encoding sequence (324-2084) selected/modified from *Eubacterium eligens* 3-isopropylmalate/(R)-2-methylmalate dehydratase large subunit (YP_002930810), a 2×84-bp *Chlamydomonas* Nia1 promoter (2085-2252), a 135-bp *Chlamydomonas* RbcS2 transit peptide (2253-2387), a 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit-encoding sequence (2388-2882) selected/modified from *Eubacterium eligens* 3-isopropylmalate/(R)-2-methylmalate dehydratase small subunit (YP_002930809), a 223-bp *Chlamydomonas* RbcS2 terminator (2883-3105), and a PCR RE primer (3106-3125).

[0304] SEQ ID NO. 136 presents example 136 for a designer Nia1-promoter-controlled chloroplast-targeted 3-Isopropylmalate Dehydratase (38) large/small subunits DNA construct (2879 bp) that includes a PCR FD primer

(sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 3-isopropylmalate dehydratase large subunit-encoding sequence (324-1727) selected/modified from *Cyanothece* 3-isopropylmalate dehydratase large subunit (YP_003886427), a 2×84-bp *Chlamydomonas* Nia1 promoter (1727-1894), a 135-bp *Chlamydomonas* RbcS2 transit peptide (1895-2029), a 3-isopropylmalate dehydratase small subunit-encoding sequence (2030-2636) selected from *Cyanothece* 3-isopropylmalate dehydratase small subunit (YP_003889452), a 223-bp *Chlamydomonas* r RbcS2 terminator (2637-2859), and a PCR RE primer (2860-2879).

[0305] SEQ ID NO. 137 presents example 137 for a designer Nia1-promoter-controlled chloroplast-targeted 3-Isopropylmalate Dehydrogenase (39) DNA construct (1661 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 3-isopropylmalate dehydrogenase-encoding sequence (324-1418) selected/modified from *Cyanothece* 3-isopropylmalate dehydrogenase (YP_003888480), a 223-bp *Chlamydomonas* RbcS2 terminator (1419-1641), and a PCR RE primer (1642-1661).

[0306] SEQ ID NO. 138 presents example 138 for a designer Nia1-promoter-controlled chloroplast-targeted 2-Isopropylmalate Synthase (40) DNA construct (2174 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 2-isopropylmalate synthase-encoding sequence (324-1931) selected/modified from *Cyanothece* 2-isopropylmalate synthase (YP_003890122), a 223-bp *Chlamydomonas* RbcS2 terminator (1932-2154), and a PCR RE primer (2155-2174).

[0307] SEQ ID NO. 139 presents example 139 for a designer Nia1-promoter-controlled chloroplast-targeted Isopropylmalate Isomerase (41) large/small subunit DNA construct (2882 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an isopropylmalate isomerase large subunit-encoding sequence (324-1727) selected/modified from *Anabaena variabilis* isopropylmalate isomerase large subunit (YP_324467), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (1728-1895), a 135-bp *Chlamydomonas* RbcS2 transit peptide (1896-2030), an isopropylmalate isomerase small subunit-encoding sequence (2031-2639) selected/modified from *Anabaena* isopropylmalate isomerase small subunit (YP_324466), a 223-bp *Chlamydomonas* RbcS2 terminator (2640-2862), and a PCR RE primer (2863-2882).

[0308] SEQ ID NO. 140 presents example 140 for a designer Nia1-promoter-controlled chloroplast-targeted 2-Keto Acid Decarboxylase (42) DNA construct (2210 bp) that includes a PCR FD primer (1-20), a 2×84-bp *Chlamydomonas* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 2-keto acid decarboxylase-encoding sequence (324-1967) selected from *Lactococcus* 2-keto acid decarboxylase (AAS49166), a 223-bp *Chlamydomonas* RbcS2 terminator (1968-2190), and a PCR RE primer (2191-2210).

[0309] SEQ ID NO. 141 presents example 141 for a designer Nia1-promoter-controlled chloroplast-targeted NADH-dependent Alcohol Dehydrogenase (43) DNA construct (1724 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nia1 promoter (21-188), a

135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a NADH-dependent alcohol dehydrogenase-encoding sequence (324-1481) selected/modified from *Gluconacetobacter hansenii* NADH-dependent alcohol dehydrogenase (ZP_06834544), a 223-bp *Chlamydomonas* RbcS2 terminator (1482-1704), and a PCR RE primer (1705-1724).

[0310] SEQ ID NO. 142 presents example 142 for a designer Nia1-promoter-controlled chloroplast-targeted NADPH-dependent Alcohol Dehydrogenase (44) DNA construct (1676 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), a NADPH-dependent alcohol dehydrogenase-encoding sequence (324-1433) selected/modified from *Fusobacterium* NADPH-dependent alcohol dehydrogenase (ZP_04573952), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (1434-1656), and a PCR RE primer (1657-1676).

[0311] Note, use of SEQ ID NOS. 129-141 (and/or 142) in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34-43/44 in FIG. 4) for photobiological production of 1-butanol from carbon dioxide and water.

[0312] SEQ ID NO. 143 presents example 143 for a designer Nia1-promoter-controlled chloroplast-targeted Phosphoenolpyruvate Carboxylase (45) DNA construct (3629 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), a Phosphoenolpyruvate Carboxylase-encoding sequence (324-3386) selected/modified from *Cyanothece* sp. PCC 7822 Phosphoenolpyruvate Carboxylase (YP_003887888), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (3387-3609), and a PCR RE primer (3610-3629).

[0313] SEQ ID NO. 144 presents example 144 for a designer Nia1-promoter-controlled chloroplast-targeted Aspartate Aminotransferase (46) DNA construct (1745 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), a Aspartate Aminotransferase-encoding sequence (324-1502) selected/modified from *Synechococcus elongatus* PCC 6301 Aspartate Aminotransferase (YP_172275), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (1503-1525), and a PCR RE primer (1526-1745).

[0314] SEQ ID NO. 145 presents example 145 for a designer Nia1-promoter-controlled chloroplast-targeted Aspartokinase (47) DNA construct (2366 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an Aspartokinase-encoding sequence (324-2123) selected/modified from *Cyanothece* Aspartokinase (YP_003136939), a 223-bp *Chlamydomonas* RbcS2 terminator (2124-2346), and a PCR RE primer (2347-2366).

[0315] SEQ ID NO. 146 presents example 146 for a designer Nia1-promoter-controlled chloroplast-targeted Aspartate-Semialdehyde Dehydrogenase (48) DNA construct (1604 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nia1 promoter

(21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an Aspartate-semialdehyde dehydrogenase-encoding sequence (324-1361) selected/modified from *Trichodesmium erythraeum* IMS101 Aspartate-semialdehyde dehydrogenase (ABG50031), a 223-bp *Chlamydomonas* RbcS2 terminator (1362-1584), and a PCR RE primer (1585-1604).

[0316] SEQ ID NO. 147 presents example 147 for a designer Nial1-promoter-controlled chloroplast-targeted Homoserine Dehydrogenase (49) DNA construct (1868 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a homoserine dehydrogenase-encoding sequence (324-1625) selected from *Cyanotheca* homoserine dehydrogenase (YP_003887242), a 223-bp *Chlamydomonas* RbcS2 terminator (1626-1848), and a PCR RE primer (1849-1868).

[0317] SEQ ID NO. 148 presents example 148 for a designer Nial1-promoter-controlled chloroplast-targeted Homoserine Kinase (50) DNA construct (1472 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a Homoserine kinase-encoding sequence (324-1229) selected/modified from *Cyanotheca* Homoserine kinase (YP_003886645), a 223-bp *Chlamydomonas* RbcS2 terminator (1230-1452), and a PCR RE primer (1453-1472).

[0318] SEQ ID NO. 149 presents example 149 for a designer Nial1-promoter-controlled chloroplast-targeted Threonine Synthase (51) DNA construct (1655 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a Threonine synthase-encoding sequence (324-1412) selected/modified from *Cyanotheca* Threonine synthase (YP_002485009), a 223-bp *Chlamydomonas* RbcS2 terminator (1413-1635), and a PCR RE primer (1636-1655).

[0319] SEQ ID NO. 150 presents example 150 for a designer Nial1-promoter-controlled chloroplast-targeted Threonine Ammonia-Lyase (52) DNA construct (2078 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a threonine ammonia-lyase-encoding sequence (324-1835) selected/modified from *Synechococcus* threonine ammonia-lyase (ZP_05035047), a 223-bp *Chlamydomonas* RbcS2 terminator (1836-2058), and a PCR RE primer (2059-2078).

[0320] Note, use of SEQ ID NOS. 129, 130, 132, 133, 143-150, 137-141 (and/or 141) through genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03, 04, 34, 35, 45-52, 39-43/44 in FIG. 4) for photobiological production of 1-butanol from carbon dioxide and water.

[0321] SEQ ID NO. 151 presents example 151 for a designer Nial1-promoter-controlled chloroplast-targeted Acetolactate Synthase (53) DNA construct (2282 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an acetolactate synthase-encoding sequence (324-2039) selected from *Bacillus subtilis* acetolactate synthase

(CAB07802), a 223-bp *Chlamydomonas* RbcS2 terminator (2040-2262), and a PCR RE primer (2263-2282).

[0322] SEQ ID NO. 152 presents example 152 for a designer Nial1-promoter-controlled chloroplast-targeted Ketol-Acid Reductoisomerase (54) DNA construct (1562 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1319) selected/modified from *Cyanotheca* ketol-acid reductoisomerase (YP_003885458), a 223-bp *Chlamydomonas* RbcS2 terminator (1320-1542), and a PCR RE primer (1543-1562).

[0323] SEQ ID NO. 153 presents example 153 for a designer Nial1-promoter-controlled chloroplast-targeted Dihydroxy-Acid Dehydratase (55) DNA construct (2252 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a dihydroxy-acid dehydratase-encoding sequence (324-2009) selected from *Cyanotheca* dihydroxy-acid dehydratase (YP_003887466), a 223-bp *Chlamydomonas* RbcS2 terminator (2010-2232), and a PCR RE primer (2233-2252).

[0324] SEQ ID NO. 154 presents example 154 for a designer Nial1-promoter-controlled chloroplast-targeted 2-Methylbutyraldehyde Reductase (56) DNA construct (1496 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1253) selected/modified from *Pichia pastoris* GS115 2-methylbutyraldehyde reductase (XP_002490018), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (1254-1476), and a PCR RE primer (1477-1496).

[0325] Note, use of SEQ ID NOS. 129-137, 140, and 151-154 in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34-39, 53-55, 42, and 56 in FIG. 5) for photobiological production of 2-methyl-1-butanol from carbon dioxide and water.

[0326] SEQ ID NO. 155 presents example 155 for a designer Nial1-promoter-controlled chloroplast-targeted 3-Methylbutanal Reductase (57) DNA construct (1595 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), a 3-methylbutanal reductase-encoding sequence (324-1352) selected/modified from *Saccharomyces cerevisiae* S288c 3-methylbutanal reductase (DAA10635), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (1353-1575), and a PCR RE primer (1576-1595).

[0327] Note, use of SEQ ID NOS. 129-133, 151-153, 140 and 141 (or 142) in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34, 35, 53-55, 42, and 43 (44) in FIG. 6) for photobiological production of isobutanol from carbon dioxide and water. Whereas, SEQ ID NOS. 129-133, 151-153, 136-138, 140 and 155 represent a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway

(03-05, 34, 35, 53-55, 40, 38, 39, 42, and 57 in FIG. 6) that can photobiologically produce 3-methyl-1-butanol from carbon dioxide and water.

[0328] SEQ ID NO. 156 presents example 156 for a designer Nial1-promoter-controlled chloroplast-targeted NADH-dependent Butanol Dehydrogenase (12a) DNA construct (1739 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1496) selected/modified from *Clostridium perfringens* NADH-dependent butanol dehydrogenase (NP_561774), a 223-bp *Chlamydomonas* RbcS2 terminator (1497-1719), and a PCR RE primer (1720-1739).

[0329] SEQ ID NO. 157 presents example 157 for a designer Nial1-promoter-controlled chloroplast-targeted NADPH-dependent Butanol Dehydrogenase (12b) DNA construct (1733 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 promoter (21-188), a 135-bp *Chlamydomonas reinhardtii* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1490) selected/modified from *Clostridium saccharobutylicum* NADPH-dependent butanol dehydrogenase (AAA83520), a 223-bp *Chlamydomonas reinhardtii* RbcS2 terminator (1491-1713), and a PCR RE primer (1714-1733).

[0330] Note, use of SEQ ID NOS. 129-140 and 156 (and/or 157) in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced butanol production pathway (03-05, 34-42 and 12 in FIG. 4) for more specific photobiological production of 1-butanol from carbon dioxide and water. Similarly, SEQ ID NOS. 129, 130, 132, 133, 143-150, 137-140, and 156 (and/or 157) represent another designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced butanol-production pathway (03, 04, 34, 35, 45-52, 39-42 and 12 in FIG. 4) for photobiological production of 1-butanol from carbon dioxide and water.

[0331] SEQ ID NO. 158 presents example 158 for a designer Nial1-promoter-controlled chloroplast-targeted 3-Ketothiolase (07') DNA construct (1745 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 (nitrate reductase) promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), a 3-Ketothiolase-encoding sequence (324-1502) selected/modified from *Azohydromonas lata* 3-Ketothiolase (AAD10275), a 223-bp *Chlamydomonas* RbcS2 terminator (1503-1725), and a PCR RE primer (1726-1745).

[0332] SEQ ID NO. 159 presents a designer Nial1-promoter-controlled chloroplast-targeted 3-Hydroxyacyl-CoA dehydrogenase (08') DNA construct (1439 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1196) selected/modified from *Oceanithermus* 3-Hydroxyacyl-CoA dehydrogenase (ADR36325), a 223-bp *Chlamydomonas* RbcS2 terminator (1197-1419), and a PCR RE primer (1420-1439).

[0333] SEQ ID NO. 160 presents example 160 for a designer Nial1-promoter-controlled chloroplast-targeted Enoyl-CoA dehydratase (09') DNA construct (1337 bp) that

includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1094) selected/modified from *Bordetella petrii* Enoyl-CoA dehydratase (YP_001629844), a 223-bp *Chlamydomonas* RbcS2 terminator (1095-1317), and a PCR RE primer (1318-1337).

[0334] SEQ ID NO. 161 presents example 161 for a designer Nial1-promoter-controlled 2-Enoyl-CoA reductase (10') DNA construct (1736 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1493) selected/modified from *Xanthomonas campestris* 2-Enoyl-CoA reductase (YP_001905744), a 223-bp *Chlamydomonas* RbcS2 terminator (1494-1716), and a PCR RE primer (1717-1736).

[0335] SEQ ID NO. 162 presents example 162 for a designer Nial1-promoter-controlled chloroplast-targeted Acyl-CoA reductase (11') DNA construct (2036 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas reinhardtii* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1793) selected/modified from *Thermosphaera aggregans* Acyl-CoA reductase (YP_003649571), a 223-bp *Chlamydomonas* RbcS2 terminator (1794-2016), and a PCR RE primer (2017-2036).

[0336] SEQ ID NO. 163 presents example 163 for a designer Nial1-promoter-controlled chloroplast-targeted Hexanol Dehydrogenase (12') DNA construct (1625 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1382) selected/modified from *Mycobacterium chubuense* hexanol dehydrogenase (ACZ56328), a 223-bp *Chlamydomonas* RbcS2 terminator (1383-1605), and a PCR RE primer (1606-1625).

[0337] Note, use of SEQ ID NOS. 158-163 with other proper DNA constructs such as SEQ ID NOS. 132 and 133 in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced hexanol production pathway (34, 35, 03-10, and 07'-12' in FIG. 7) for photobiological production of 1-hexanol from carbon dioxide and water.

[0338] SEQ ID NO. 164 presents example 164 for a designer Nial1-promoter-controlled chloroplast-targeted Octanol Dehydrogenase (12'') DNA construct (1249 bp) that includes a PCR FD primer (sequence 1-20), a 2×84-bp *Chlamydomonas* Nial1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1006) selected/modified from *Drosophila subobscura* Octanol dehydrogenase (ABO65263), a 223-bp *Chlamydomonas* RbcS2 terminator (1007-1229), and a PCR RE primer (1230-1249).

[0339] Note, SEQ ID NOS. 132, 133, and 158-163 represent a designer eukaryotic photosynthetic organism such as a designer *Chlamydomonas* with a designer hydrocarbon chain elongation pathway (34, 35, 07'-12' as shown in FIG. 7) for photobiological production of 1-hexanol. SEQ ID NOS. 132, 133, 158-162 and 164 represent another designer eukaryotic photosynthetic organism such as a designer *Chlamydomonas*

with a designer hydrocarbon chain elongation pathway (34, 35, 07'-10' and 07''-12'' as shown in FIG. 7) for photobiological production of 1-octanol.

[0340] SEQ ID NO. 165: a designer Nia1-promoter-controlled chloroplast-targeted Short Chain Alcohol Dehydrogenase (43') DNA construct (1769 bp) that includes a PCR FD primer (sequence 1-20), a 2x84-bp *Chlamydomonas* Nia1 promoter (21-188), a 135-bp *Chlamydomonas* RbcS2 transit peptide (189-323), an enzyme-encoding sequence (324-1526) selected/modified from *Burkholderia* Short chain alcohol dehydrogenase (AB056626), a 223-bp *Chlamydomonas* RbcS2 terminator (1527-1749), and a PCR RE primer (1750-1769).

[0341] Note, use of SEQ ID NOS. 129-140 and 165 in genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34-41, 39'-43', 39''-43'' and 39'''-43''' in FIG. 8) for photobiological production of 1-pentanol, 1-hexanol, and 1-heptanol from carbon dioxide and water. Similarly, SEQ ID NOS. 129-140 and 163 represent another designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34-41, 39'-41', 39'-42' and 12' in FIG. 8) for photobiological production of 1-hexanol from carbon dioxide and water.

[0342] Likewise, use of SEQ ID NOS. 129-137, 151-153, 138-140 and 165 through genetic transformation of an eukaryotic photosynthetic organism such as *Chlamydomonas* can create a designer eukaryotic photosynthetic organism such as designer *Chlamydomonas* with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34-39, 53-55, 39'-43', 39''-43'', and 39'''-43''' in FIG. 9) for photobiological production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and 5-methyl-1-heptanol from carbon dioxide and water; The expression of SEQ ID NOS. 129, 130, 132, 133, 143-150, 151-153, 137-140 and 165 in an eukaryotic photosynthetic organism such as a host *Chlamydomonas* represent another designer eukaryotic photosynthetic organism with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03, 05, 34, 35, 42-55, 39'-43', 39''-43'', and 39'''-43''' in FIG. 9) for photobiological production of 3-methyl-1-pentanol, 4-methyl-1-hexanol, and 5-methyl-1-heptanol from carbon dioxide and water; The expression of SEQ ID NOS. 129-133, 151-153, 136-140 and 165 in a host eukaryotic photosynthetic organism such as *Chlamydomonas* represent yet another designer eukaryotic photosynthetic organism with a Calvin-cycle 3-phosphoglycerate-branched NADPH-enhanced pathway (03-05, 34, 35, 53-55, 40, 38, 39, 39'-43', 39''-43'', and 39'''-43''' in FIG. 10) for photobiological production of 4-methyl-1-pentanol, 5-methyl-1-hexanol, and 6-methyl-1-heptanol from carbon dioxide and water.

Use of Designer Photosynthetic Organisms with Photobioreactor for Production and Harvesting of Butanol and Related Higher Alcohols

[0343] The designer photosynthetic organisms with designer Calvin-cycle channeled photosynthetic NADPH-enhanced pathways (FIGS. 1, and 4-10) can be used with photobioreactors for production and harvesting of butanol and/or related higher alcohols. The said butanol and/or related higher alcohols are selected from the group consisting of: 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-

butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, 6-methyl-1-heptanol, and combinations thereof.

[0344] The said designer photosynthetic organisms such as designer transgenic oxyphotobacteria and algae comprise designer Calvin-cycle-channeled and photosynthetic NADPH-enhanced pathway gene(s) and biosafety-guarding technology for enhanced photobiological production of butanol and related higher alcohols from carbon dioxide and water. According to one of the various embodiments, it is a preferred practice to grow designer photosynthetic organisms photoautotrophically using carbon dioxide (CO₂) and water (H₂O) as the sources of carbon and electrons with a culture medium containing inorganic nutrients. The nutrient elements that are commonly required for oxygenic photosynthetic organism growth are: N, P, and K at the concentrations of about 1-10 mM, and Mg, Ca, S, and Cl at the concentrations of about 0.5 to 1.0 mM, plus some trace elements Mn, Fe, Cu, Zn, B, Co, Mo among others at μM concentration levels. All of the mineral nutrients can be supplied in an aqueous minimal medium that can be made with well-established recipes of oxygenic photosynthetic organism (such as algal) culture media using water (freshwater for the designer freshwater algae; seawater for the salt-tolerant designer marine algae) and relatively small of inexpensive fertilizers and mineral salts such as ammonium bicarbonate (NH₄HCO₃) (or ammonium nitrate, urea, ammonium chloride), potassium phosphates (K₂HPO₄ and KH₂PO₄), magnesium sulfate heptahydrate (MgSO₄·7H₂O), calcium chloride (CaCl₂), zinc sulfate heptahydrate (ZnSO₄·7H₂O), iron (II) sulfate heptahydrate (FeSO₄·7H₂O), and boric acid (H₃BO₃), among others. That is, large amounts of designer algae (or oxyphotobacteria) cells can be inexpensively grown in a short period of time because, under aerobic conditions such as in an open pond, the designer algae can photoautotrophically grow by themselves using air CO₂ as rapidly as their wild-type parental strains. This is a significant feature (benefit) of the invention that could provide a cost-effective solution in generation of photoactive biocatalysts (the designer photosynthetic biofuel-producing organisms such as designer algae or oxyphotobacteria) for renewable solar energy production.

[0345] According to one of the various embodiments, when designer photosynthetic organism culture is grown and ready for photobiological production of butanol and/or related higher alcohols, the designer photosynthetic organism cells are then induced to express the designer Calvin-cycle channeled photosynthetic NADPH-enhanced pathway(s) to photobiologically produce butanol and/or related higher alcohols from carbon dioxide and water. The method of induction is designer pathway gene(s) specific. For example, if/when a *nirA* promoter is used to control the designer Calvin-cycle channeled pathway gene(s) such as those of SEQ ID NOS: 58-69 and 72 (and/or 73) which represent a designer transgenic *Thermosynechococcus* that comprises the designer genes of a Calvin-cycle 3-phosphoglycerate-branched photosynthetic NADPH-enhanced pathway (numerically labeled as 34, 35, 03-05, 36-42, and 12 in FIG. 4) for photobiological production of 1-butanol from carbon dioxide and water, the designer transgenic *Thermosynechococcus* is grown in a minimal liquid culture medium containing ammonium (but no nitrate) and other inorganic nutrients. When the designer transgenic *Thermosynechococcus* culture is grown and ready for photobiological production of biofuel 1-butanol, nitrate

fertilizer will then be added into the culture medium to induce the expression of the designer *nirA*-controlled Calvin-cycle-channelled pathway to photobiologically produce 1-butanol from carbon dioxide and water in this example.

[0346] For the designer photosynthetic organism(s) with anaerobic promoter-controlled pathway(s) such as the designer transgenic *Nostoc* that contains designer *hox*-promoter-controlled Calvin-cycle 3-phosphoglycerate-branched pathway genes of SEQ ID NOS. 104-109, anaerobic conditions can be used to induce the expression of the designer pathway gene(s) for photobiological production of 2-methyl-1-butanol from carbon dioxide and water (FIG. 5). That is, when the designer transgenic *Nostoc* culture is grown and ready for photobiological biofuel production, its cells will then be placed (or sealed) into certain anaerobic conditions to induce the expression of the designer *hox*-controlled pathway gene(s) to photobiologically produce 2-methyl-1-butanol from carbon dioxide and water.

[0347] For those designer photosynthetic organism(s) that contains a heat- and light-responsive promoter-controlled and *nirA*-promoter-controlled pathway(s) such as the designer transgenic *Prochlorococcus* that contains a set of designer *groE*-promoter-controlled and *nirA*-promoter-controlled Calvin-cycle 3-phosphoglycerate-branched pathway genes of SEQ ID NOS. 110-118, light and heat are used in conjunction of nitrate addition to induce the expression of the designer pathway genes for photobiological production of isobutanol from carbon dioxide and water (FIG. 6).

[0348] According to another embodiment, use of designer marine algae or marine oxyphotobacteria enables the use of seawater and/or groundwater for photobiological production of biofuels without requiring freshwater or agricultural soil. For example, designer *Prochlorococcus marinus* that contains the designer genes of SEQ ID NOS: 110-117 and 119-122 can use seawater and/or certain groundwater for photoautotrophic growth and synthesis of 3-methyl-1-butanol from carbon dioxide and water with its *groE* promoter-controlled designer Calvin-cycle-channelled pathway (identified as 34 (native), 35, 03-05, 53-55, 38-40, 42 and 57 in FIG. 6). The designer photosynthetic organisms can be used also in a sealed photobioreactor that is operated on a desert for production of isobutanol with highly efficient use of water since there will be little or no water loss by evaporation and/or transpiration that a common crop system would suffer. That is, this embodiment may represent a new generation of renewable energy (butanol and related higher alcohols) production technology without requiring arable land or freshwater resources.

[0349] According to another embodiment, use of nitrogen-fixing designer oxyphotobacteria enables photobiological production of biofuels without requiring nitrogen fertilizer. For example, the designer transgenic *Nostoc* that contains designer *hox*-promoter-controlled genes of SEQ ID NOS. 104-109 is capable of both fixing nitrogen (N_2) and photobiologically producing 2-methyl-1-butanol from carbon dioxide and water (FIG. 6). Therefore, use of the designer transgenic *Nostoc* enables photoautotrophic growth and 2-methyl-1-butanol synthesis from carbon dioxide and water.

[0350] Certain designer oxyphotobacteria are designed to perform multiple functions. For example, the designer transgenic *Cyanothece* that contains designer *nirA* promoter-controlled genes of SEQ ID NOS. 123-127 is capable of (1) using seawater, (2) N_2 fixing nitrogen, and photobiological producing 1-hexanol from carbon dioxide and water (FIG. 8). Use of

this type of designer oxyphotobacteria enables photobiological production of advanced biofuels such as 1-hexanol using seawater without requiring nitrogen fertilizer

[0351] According to one of various embodiments, a method for photobiological production and harvesting of butanol and related higher alcohols comprises: a) introducing a transgenic photosynthetic organism into a photobiological reactor system, the transgenic photosynthetic organism comprising transgenes coding for a set of enzymes configured to act on an intermediate product of a Calvin cycle and to convert the intermediate product into butanol and related higher alcohols; b) using reducing power and energy associated with the transgenic photosynthetic organism acquired from photosynthetic water splitting and proton gradient coupled electron transport process in the photobioreactor to synthesize butanol and related higher alcohols from carbon dioxide and water; and c) using a product separation process to harvest the synthesized butanol and/or related higher alcohols from the photobioreactor.

[0352] In summary, there are a number of embodiments on how the designer organisms may be used for photobiological butanol (and/or related higher alcohols) production. One of the preferred embodiments is to use the designer organisms for direct photosynthetic butanol production from CO_2 and H_2O with a photobiological reactor and butanol-harvesting (filtration and distillation/evaporation) system, which includes a specific operational process described as a series of the following steps: a) Growing a designer transgenic organism photoautotrophically in minimal culture medium using air CO_2 as the carbon source under aerobic (normal) conditions before inducing the expression of the designer butanol-production-pathway genes; b) When the designer organism culture is grown and ready for butanol production, sealing or placing the culture into a specific condition to induce the expression of designer Calvin-cycle-channelled pathway genes; c) When the designer pathway enzymes are expressed, supplying visible light energy such as sunlight for the designer-genes-expressed cells to work as the catalysts for photosynthetic production of butanol and/or related higher alcohols from CO_2 and H_2O ; d) Harvesting the product butanol and/or related higher alcohols by any method known to those skilled in the art. For example, harvesting the butanol and/or related higher alcohols from the photobiological reactor can be achieved by a combination of membrane filtration and distillation/evaporation butanol-harvesting techniques.

[0353] The above process to use the designer organisms for photosynthetic production and harvesting of butanol and related higher alcohols can be repeated for a plurality of operational cycles to achieve more desirable results. Any of the steps a) through d) of this process described above can also be adjusted in accordance of the invention to suit for certain specific conditions. In practice, any of the steps a) through d) of the process can be applied in full or in part, and/or in any adjusted combination as well for enhanced photobiological production of butanol and higher alcohol in accordance of this invention.

[0354] In addition to butanol and/or related higher alcohols production, it is also possible to use a designer organism or part of its designer butanol-production pathway(s) to produce certain intermediate products of the designer Calvin-cycle-channelled pathways (FIGS. 1 and 4-10) including (but not limited to): butyraldehyde, butyryl-CoA, crotonyl-CoA, 3-hydroxybutyryl-CoA, acetoacetyl-CoA, acetyl-CoA, pyruvate, phosphoenolpyruvate, 2-phosphoglycerate, 1,3-diphos-

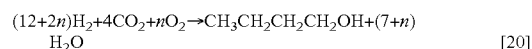
phoglycerate, glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, fructose-1,6-diphosphate, fructose-6-phosphate, glucose-6-phosphate, glucose, glucose-1-phosphate, citramalate, citraconate, methyl-D-malate, 2-ketobutyrate, 2-ketovalerate, oxaloacetate, aspartate, homoserine, threonine, 2-keto-3-methylvalerate, 2-methylbutyraldehyde, 3-methylbutyraldehyde, 4-methyl-2-oxopentanoate, 3-isopropylmalate, 2-isopropylmalate, 2-oxoisovalerate, 2,3-dihydroxyisovalerate, 2-acetolactate, isobutyraldehyde, 3-keto-C6-acyl-CoA, 3-hydroxy-C6-acyl-CoA, C6-enoyl-CoA, C6-acyl-CoA, 3-keto-C8-acyl-CoA, 3-hydroxy-C8-acyl-CoA, C8-enoyl-CoA, C8-acyl-CoA, octanal, 1-pentanol, 1-hexanal, 1-heptanal, 2-ketohexanoate, 2-ketoheptanoate, 2-ketooctanoate, 2-ethylmalate, 3-ethylmalate, 3-methyl-1-pentanal, 4-methyl-1-hexanal, 5-methyl-1-heptanal, 2-hydroxy-2-ethyl-3-oxobutanoate, 2,3-dihydroxy-3-methyl-pentanoate, 2-keto-4-methyl-hexanoate, 2-keto-5-methyl-heptanoate, 2-keto-6-methyl-octanoate, 4-methyl-1-pentanal, 5-methyl-1-hexanal, 6-methyl-1-heptanal, 2-keto-7-methyl-octanoate, 2-keto-6-methyl-heptanoate, and 2-keto-5-methyl-hexanoate. According to one of various embodiments, therefore, a further embodiment comprises an additional step of harvesting the intermediate products that can be produced also from an induced transgenic designer organism. The production of an intermediate product can be selectively enhanced by switching off a designer-enzyme activity that catalyzes its consumption in the designer pathways. The production of a said intermediate product can be enhanced also by using a designer organism with one or some of designer enzymes omitted from the designer butanol-production pathways. For example, a designer organism with the butanol dehydrogenase or butyraldehyde dehydrogenase omitted from the designer pathway(s) of FIG. 1 may be used to produce butyraldehyde or butyryl-CoA, respectively.

Designer Calvin-Cycle-Channeled Aerobic Hydrogenotrophic Biofuel Pathways

[0355] According to one of the various embodiments, a designer hydrogenotrophic Calvin-cycle-channeled pathway technology (FIG. 11) is created that takes hydrogen (H₂), oxygen (O₂) and carbon dioxide (CO₂) to produce advanced biofuels including butanol and related higher alcohols through the designer Calvin-cycle-channeled pathways (FIGS. 1 and 4-10). As illustrated in FIG. 11, one of the various embodiments here is the expression of designer oxygen (O₂)-tolerant hydrogenases in a designer microbial cell such as cyanobacteria to generate NAD(P)H and ATP from consumption of hydrogen. The expression of a membrane bound hydrogenase (MBH, 70 and its accessory proteins 72 as listed in Table 1) enables oxidation of H₂ through the respiratory electron transport chain (ETC) system to pump protons (H⁺) across the cytoplasm membrane to create trans-membrane electrochemical potential for ATP synthesis; whereas the use of a soluble hydrogenase (SH, 71 and its accessory proteins 72) enables generation of NAD(P)H through SH-mediated reduction of NAD(P)⁺ by H₂. Use of ATP and NAD(P)H drives the designer Calvin-cycle-channeled pathways (FIGS. 1 and 4-10) for CO₂ fixation and biofuel butanol and related higher alcohol production. Therefore, this represents an innovative application of the designer Calvin-cycle-channeled biofuel-production pathways.

[0356] For example, the expression of a membrane bound hydrogenase (MBH, 70 and its accessory proteins 72) and a soluble hydrogenase (SH, 71 and its accessory proteins 72) in

a designer transgenic cyanobacterium that already contains the designer butanol-production-pathway genes of SEQ ID NOS: 58-69 and 72 (and/or 73) can create a hydrogenotrophic Calvin-cycle 3-phosphoglycerate-branched 1-butanol production pathway as numerically labeled as 34, 35, 03-05, 36-42, and 12 in FIG. 4. The net result of the designer hydrogenotrophic pathway is the production of 1-butanol (CH₃CH₂CH₂CH₂OH) from hydrogen (H₂), carbon dioxide (CO₂) and oxygen (O₂) according to the following process reaction:



The number (n) of oxygen (O₂) molecules used to oxidize hydrogen (H₂) by the respiratory electron-transport-coupled phosphorylation to support the synthesis of a 1-butanol was estimated to be about 5 in this example.

[0357] Note, before the designer genes are turned on, the transgenic cyanobacteria (FIG. 11) can grow photoautotrophically using CO₂, H₂O and sunlight just like their wild-type parental strains. When they are grown and ready for use, they can then be placed into a bioreactor supplied with H₂ (about 85%) and CO₂ (about 10%) with limiting amount of O₂ (about 5%) for hydrogenotrophic synthesis of higher alcohols such as 1-butanol, for example, through the Calvin-cycle-channeled butanol-production pathway of FIG. 1 without requiring any photosynthesis or sunlight. Since hydrogen (H₂) can be made from a number of sources including the electrolysis of water, the designer hydrogenotrophic Calvin-cycle-channeled pathway technology (FIG. 11) enables utilization of inexpensive industrial CO₂ and electricity from solar photovoltaic, wind and nuclear power stations to produce “drop-in-ready” liquid transportation fuel such as butanol without requiring any arable lands or photosynthesis.

Designer Anaerobic Hydrogenotrophic Reductive-Acetyl-CoA Biofuel-Production Pathways

[0358] According to one of the various embodiments, a designer hydrogenotrophic reductive-acetyl-CoA biofuel-production pathway technology (FIG. 12) is created that takes hydrogen (H₂) and carbon dioxide (CO₂) to produce advanced biofuels such as butanol and related higher alcohols under anaerobic conditions. As illustrated in FIG. 12, one of the various embodiments here is the expression of a set of designer genes that confer a designer anaerobic hydrogenotrophic system and a reductive-acetyl-CoA butanol-producing pathway (FIG. 13) in a microbial host cell such as a cyanobacterium. Designer anaerobic hydrogenotrophic system includes, for example, energy converting hydrogenase (Ech, 91 in Table 1), [NiFe]-hydrogenase Mvh (95), Coenzyme F₄₂₀-reducing hydrogenase (Frh, 96), native (or heterologous) soluble hydrogenase (SH, 71), NAD(P)H, reduced ferredoxin (Fd_{red}²⁻), HS-CoM, HS-CoB, and heterodisulfide reductase (Hdr; 94); while designer reductive-acetyl-CoA butanol-producing pathway (as shown with the numerical labels 83-90 and 07-12/43 in FIG. 13) comprises formylmethanofuran dehydrogenase 83, formyl transferase 84, 10-methenyl-tetrahydromethanopterin cyclohydrolase 85, 10-methylene-H₄ methanopterin dehydrogenase 86, 10-methylene-H₄-methanopterin reductase 87, methyl-H₄-methanopterin: corrinoid iron-sulfur protein methyltransferase 88, corrinoid iron-sulfur protein 89, CO dehydrogenase/acetyl-CoA synthase 90, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA

dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12, and/or alcohol dehydrogenase 43. In this example, the net result of the designer anaerobic hydrogenotrophic reductive-acetyl-CoA butanol-production pathway technology (FIGS. 12 and 13) is the production of 1-butanol ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$) from hydrogen (H_2) and carbon dioxide (CO_2) according to the following process reaction:



The standard free energy change ($\Delta_r G^\circ$) for this overall reaction is -244.7 kJ/mol 1-butanol, which demonstrates that this hydrogen-driven butanol-production technology is not in violation of thermodynamic laws. This equation shows that the use of 12 molecules (24 electrons) of hydrogen (H_2) can produce one molecule of 1-butanol from 4 molecules of carbon dioxide (CO_2). To produce 12 molecules of H_2 by electrolysis of water, it uses 24 electrons from electricity. Therefore, if electrolysis of water is used as a hydrogen source, then 24 electrons (from electricity) are sufficient to generate one molecule of 1-butanol from 4 molecules of CO_2 through the designer anaerobic hydrogenotrophic reductive-acetyl-CoA butanol-production pathway technology (FIGS. 12 and 13).

[0359] Therefore, in one of the various embodiments, a designer autotrophic organism comprises a set of designer genes (e.g., designer DNA constructs) that express a set of enzymes conferring the designer anaerobic hydrogenotrophic butanol-production-pathway system (as shown in FIGS. 12 and 13) that comprises: energy converting hydrogenase (Ech) 91, [NiFe]-hydrogenase (Mvh) 95, Coenzyme F_{420} -reducing hydrogenase (Frh) 96, native (or heterologous) soluble hydrogenase (SH) 71, heterodissulfide reductase (Hdr) 94, formylmethanofuran dehydrogenase 83, formyl transferase 84, 10-methenyl-tetrahydromethanopterin cyclohydrolase 85, 10-methylene- H_4 methanopterin dehydrogenase 86, 10-methylene- H_4 -methanopterin reductase 87, methyl- H_4 -methanopterin: corrinoide iron-sulfur protein methyltransferase 88, corrinoide iron-sulfur protein 89, CO dehydrogenase/acetyl-CoA synthase 90, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12 and/or alcohol dehydrogenase 43.

[0360] Before the designer genes are turned on, the designer transgenic cyanobacteria (FIG. 12) can grow photoautotrophically using CO_2 , H_2O and sunlight just like their wild-type parental strains. When they are grown and ready for use, they can then be placed into a bioreactor for butanol production from H_2 and CO_2 under anaerobic conditions without requiring any photosynthesis or any respiratory oxidation of H_2 by molecular oxygen (O_2). A unique feature of this designer reductive-acetyl-CoA butanol-production pathway (FIG. 13) is that it does not require any ATP; this pathway uses reduced ferredoxin (Fd_{red}^{2-}), F_{420}H_2 and NAD(P)H that the designer anaerobic hydrogenotrophic system (FIG. 12) can supply from H_2 employing certain electro-proton-coupled bioenergetics bifurcating mechanism. In accordance with one of the various embodiments, this designer pathway (FIG. 13) represents one of the most energy-efficient butanol-production processes identified so far. The standard free energy change ($\Delta_r G^\circ$) of this specific anaerobic hydrogenotrophic butanol-production process [Eq. 21] is -20.4 kJ/mol per H_2 used. Its maximum hydrogen (H_2)-to-butanol energy conversion efficiency was estimated to be about 91.4%.

[0361] According to one of the various embodiments, another designer anaerobic reductive-acetyl-CoA butanol-production pathway (as shown with the numerical labels 74-81 and 07-12/43 in FIG. 14) is created that can produce 1-butanol from H_2 and CO_2 through use of a set of enzymes comprising: formate dehydrogenase 74, 10-formyl- H_4 folate synthetase 75, methenyltetrahydrofolate cyclohydrolase 76, 10-methylene- H_4 folate dehydrogenase 77, 10-methylene- H_4 folate reductase 78, methyl- H_4 folate: corrinoide iron-sulfur protein methyltransferase 79, corrinoide iron-sulfur protein 80, CO dehydrogenase/acetyl-CoA synthase 81, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12, and/or alcohol dehydrogenase 43.

[0362] This designer pathway is similar to that of FIG. 13, except that it requires consumption of ATP at the step of 10-formyl- H_4 folate synthetase 75 (FIG. 14). Therefore, it requires ATP supply from other cellular processes in order to operate. According to one of the various embodiments, this pathway (FIG. 14) can be supported by a designer methanogenic hydrogenotrophic cell system (FIG. 15) that produces ATP, Fd_{red}^{2-} , F_{420}H_2 , and NAD(P)H. This designer autotrophic organism comprises a set of designer genes (e.g., designer DNA constructs) that express the designer methanogenic hydrogenotrophic butanol-production-pathway system (as shown in FIGS. 14 and 16) comprising: methyl- H_4MPT : coenzyme-M methyltransferase Mtr 92, native (or heterologous) A_1A_o -ATP synthase 97, methyl-coenzyme M reductase Mcr 93, energy converting hydrogenase (Ech) 91, [NiFe]-hydrogenase (Mvh) 95, Coenzyme F_{420} -reducing hydrogenase (Frh) 96, native (or heterologous) soluble hydrogenase (SH) 71, heterodissulfide reductase (Hdr) 94, formylmethanofuran dehydrogenase 83, formyl transferase 84, 10-methenyl-tetrahydromethanopterin cyclohydrolase 85, 10-methylene- H_4 methanopterin dehydrogenase 86, 10-methylene- H_4 -methanopterin reductase 87, methyl- H_4 -methanopterin: corrinoide iron-sulfur protein methyltransferase 88, corrinoide iron-sulfur protein 89, CO dehydrogenase/acetyl-CoA synthase 90, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12 and/or alcohol dehydrogenase 43.

[0363] For example, the designer methanogenic hydrogenotrophic system (FIG. 15) comprises methyl- H_4MPT : coenzyme-M methyltransferase Mtr 92, A_1A_o -ATP synthase 97, energy converting hydrogenase (Ech; 91 in Table 1), [NiFe]-hydrogenase Mvh (95), Coenzyme F_{420} -reducing hydrogenase (Frh, 96), native (or heterologous) soluble hydrogenase (SH, 71), NAD(P)H, reduced ferredoxin (Fd_{red}^{2-}), HS-CoM, HS-CoM, methyl-coenzyme M reductase Mcr 93, and heterodissulfide reductase (Hdr, 94). The Mtr 92 in this system can take a fraction of the $\text{CH}_3\text{—H}_4\text{MPT}$ intermediate to produce methane and generate a transmembrane electrochemical potential for synthesis of ATP, which can support the ATP-requiring anaerobic reductive-acetyl-CoA butanol-production pathway of FIG. 14. Therefore, the combination of the methanogenic hydrogenotrophic system (FIG. 15) and the ATP-requiring anaerobic reductive-acetyl-CoA butanol-production pathway (FIG. 14) results in a combined pathway (FIG. 16) for production of both butanol and methane. The net result is the production of both butanol and methane (CH_4) from hydrogen (H_2) and carbon dioxide

(CO₂) according to the following process reaction where m is the number of CH₄ molecules co-generated per 1-butanol produced:



[0364] The non-ATP-requiring anaerobic reductive-acetyl-CoA butanol-production pathway (FIG. 13) can, of course, operate with this designer methanogenic hydrogenotrophic system (FIG. 15) as well, resulting in another combined pathway for production of both butanol and methane (FIG. 17). Therefore, in one of the various embodiments, a designer autotrophic organism comprises a set of designer genes (e.g., designer DNA constructs) that express a designer methanogenic hydrogenotrophic butanol-production-pathway system (as shown in FIGS. 15, 13, and 17) comprising: methyl-H4MPT: coenzyme-M methyltransferase Mtr 92, native (or heterologous) A₁A_o-ATP synthase 97, methyl-coenzyme M reductase Mcr 93, energy converting hydrogenase (Ech) 91, [NiFe]-hydrogenase (Mvh) 95, Coenzyme F₄₂₀-reducing hydrogenase (Frh) 96, native (or heterologous) soluble hydrogenase (SH) 71, heterodissulfide reductase (Hdr) 94, formate dehydrogenase 74, 10-formyl-H₄ folate synthetase 75, methenyltetrahydrofolate cyclohydrolase 76, 10-methylene-H₄ folate dehydrogenase 77, 10-methylene-H₄ folate reductase 78, methyl-H₄ folate: corrinoid iron-sulfur protein methyltransferase 79, corrinoid iron-sulfur protein 80, CO dehydrogenase/acetyl-CoA synthase 81, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12, and/or alcohol dehydrogenase 43.

[0365] Some of these enzymes may naturally exist in some of the host organisms depending on their genetic background; some of these native enzymes may be used in constructing part of the designer pathways (FIGS. 12-17) along with designer genes. Therefore, according to one of the various embodiments, a designer autotrophic organism for production of biofuels such as butanol through anaerobic hydrogenotrophic reductive-acetyl-CoA biofuel-production-pathway(s) comprises designer genes that can express at least one of the enzymes selected from the group consisting of: energy converting hydrogenase (Ech) 91, methyl-H4MPT: coenzyme-M methyltransferase Mtr 92, methyl-coenzyme M reductase Mcr 93, heterodissulfide reductase (Hdr) 94, [NiFe]-hydrogenase (Mvh) 95, Coenzyme F₄₂₀-reducing hydrogenase (Frh) 96, soluble hydrogenase (SH) 71, A₁A_o-ATP synthase 97, formate dehydrogenase 74, 10-formyl-H₄ folate synthetase 75, methenyltetrahydrofolate cyclohydrolase 76, 10-methylene-H₄ folate dehydrogenase 77, 10-methylene-H₄ folate reductase 78, methyl-H₄ folate: corrinoid iron-sulfur protein methyltransferase 79, corrinoid iron-sulfur protein 80, CO dehydrogenase/acetyl-CoA synthase 81, formylmethanofuran dehydrogenase 83, formyl transferase 84, 10-methenyl-tetrahydromethanopterin cyclohydrolase 85, 10-methylene-H₄ methanopterin dehydrogenase 86, 10-methylene-H₄-methanopterin reductase 87, methyl-H₂-methanopterin: corrinoid iron-sulfur protein methyltransferase 88, corrinoid iron-sulfur protein 89, CO dehydrogenase/acetyl-CoA synthase 90, thiolase 07, 3-hydroxybutyryl-CoA dehydrogenase 08, crotonase 09, butyryl-CoA dehydrogenase 10, butyraldehyde dehydrogenase 11, butanol dehydrogenase 12 and/or alcohol dehydrogenase 43.

[0366] SEQ ID NOS. 166-198 present examples for designer DNA constructs of designer enzymes for creation of designer hydrogenotrophic biofuel-producing organisms

such as designer cyanobacteria with reductive-acetyl-CoA biofuel-production pathways. Briefly, SEQ ID NO: 166 presents example 166 of a designer hox-promoter-controlled Formylmethanofuran dehydrogenase (Fmd; 83) DNA construct (6110 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-5659) selected/modified from the sequence of formylmethanofuran dehydrogenase subunits B, C, E (GenBank: ADL58895, ADL58894, ADL58893) of *Methanothermobacter marburgensis* and formylmethanofuran dehydrogenase subunits subunits A, D, and G (GenBank: ABC56660, ABC56658, ABC56657) of *Methanosphaera stadtmanae*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (5659-6090), and a PCR RE primer (6091-6110) at the 3' end.

[0367] SEQ ID NO: 167 presents example 167 of a designer hox-promoter-controlled Formyl transferase (84) DNA construct (1538 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1086) selected/modified from the sequence of a formylmethanofuran-tetrahydromethanopterin formyltransferase (GenBank: ADL59225) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc* gor terminator (1087-1518), and a PCR RE primer (1519-1538).

[0368] SEQ ID NO: 168 presents example 168 of a designer hox-promoter-controlled 5,10-Methenyl-tetrahydromethanopterin (H₄ methanopterin) cyclohydrolase (85) DNA construct (1631 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-1179) selected from the sequence of a N(5),N(10)-methenyltetrahydromethanopterin cyclohydrolase (GenBank: ABC57615) of *Methanosphaera stadtmanae*, a 432-bp *Nostoc* gor terminator (1180-1161), and a PCR RE primer (1162-1631).

[0369] SEQ ID NO: 169 presents example 169 of a designer hox-promoter-controlled 5,10-Methylene-H₄-methanopterin dehydrogenase (86) DNA construct (1475 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-1023) selected from the sequence of a F₄₂₀-dependent methylene-5,6,7,8-tetrahydromethanopterin dehydrogenase (GenBank: ADL57660) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc* gor terminator (1023-1455), and a PCR RE primer (1456-1475).

[0370] SEQ ID NO: 170 presents example 170 of a designer hox-promoter-controlled Methylenetetrahydrofolate reductase and/or Methylene-H₄-methanopterin reductase (78, 87) DNA construct (2594 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-2142) selected/modified from the sequence of a methylenetetrahydrofolate reductase (GenBank: YP_430048) of *Moorella thermoacetica* and a coenzyme F₄₂₀-dependent N(5),N(10)-methenyltetrahydromethanopterin reductase (GenBank: ADN36752) of *Methanoplanus petrolearius*, a 432-bp *Nostoc* gor terminator (2143-2574), and a PCR RE primer (2575-2594).

[0371] SEQ ID NO: 171 presents example 171 of a designer hox-promoter-controlled Methyltetrahydrofolate:corrinoid/iron-sulfur protein methyltransferase (79, 88) DNA construct (2819 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-2467) selected/

modified from the sequence of a methyltetrahydrofolate:corrinoid/iron-sulfur protein methyltransferase (GenBank: YP_430950) of *Moorella thermoacetica*, and acetyl-CoA decarboxylase/synthase, subunit gamma (GenBank: ADL57900) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (2468-2899), and a PCR RE primer (2900-2819).

[0372] SEQ ID NO: 172 presents example 172 of a designer hox-promoter-controlled Corrinoid iron-sulfur protein (80, 89) DNA construct (2771 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-2319) selected/modified from the sequence of a small subunit corrinoid iron-sulfur protein (GenBank: AAA23255) of *Moorella thermoacetica*, and acetyl-CoA decarboxylase/synthase subunit delta (GenBank: ADL57899) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc gor* terminator (2319-2751), and a PCR RE primer (2752-2771).

[0373] SEQ ID NO: 173 presents example 173 of a designer hox-promoter-controlled CO dehydrogenase/acetyl-CoA synthase (81, 90) DNA construct (7061 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-6609) selected/modified from the sequence of acetyl-CoA decarboxylase/synthase beta subunit/acetyl-CoA decarboxylase/synthase alpha subunit (GenBank: ABC19516) of *Moorella thermoacetica*, and acetyl-CoA decarboxylase/synthase subunits alpha, beta, epsilon (GenBank: ADL57895, ADL59006, ADL57897) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (6610-7041), and a PCR RE primer (7042-7061).

[0374] SEQ ID NO: 174 presents example 174 of a designer hox-promoter-controlled Thiolase (07) DNA construct (1847 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1395) selected/modified from the sequence of thiolase (GenBank: AB190764) of *Butyrivibrio fibrisolvens*, a 432-bp *Nostoc gor* terminator (1396-1827), and a PCR RE primer (1828-1847).

[0375] SEQ ID NO: 175 presents example 175 of a designer hox-promoter-controlled 3-Hydroxybutyryl-CoA dehydrogenase (08) DNA construct (1514 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1062) selected/modified from the sequence of 3-hydroxybutyryl coenzyme A dehydrogenase (GenBank: Z92974) of *Thermoanaerobacterium*, a 432-bp *Nostoc gor* terminator (1063-1494), and a PCR RE primer (1495-1514).

[0376] SEQ ID NO: 176 presents example 176 of a designer hox-promoter-controlled Crotonase (09) DNA construct (1430 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-978) selected from the sequence of crotonase (GenBank: AF494018) of *Clostridium beijerinckii*, a 432-bp *Nostoc gor* terminator (979-1410), and a PCR RE primer (1411-1430).

[0377] SEQ ID NO: 177 presents example 177 of a designer hox-promoter-controlled Butyryl-CoA dehydrogenase (10) DNA construct (1784 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1332) selected/modified from the sequence of butyryl-CoA

dehydrogenase (GenBank: AF494018) of *Clostridium beijerinckii*, a 432-bp *Nostoc gor* terminator (1333-1764), and a PCR RE primer (1765-1784).

[0378] SEQ ID NO: 178 presents example 178 of a designer hox-promoter-controlled Butyraldehyde dehydrogenase (11) DNA construct (2051 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1599) selected/modified from the sequence of butyraldehyde dehydrogenase (GenBank: AY251646) of *Clostridium saccharoperbutylacetonicum*, a 432-bp *Nostoc gor* terminator (1600-2031), and a PCR RE primer (2032-2051).

[0379] SEQ ID NO: 179 presents example 179 of a designer hox-promoter-controlled NADH-dependent Butanol dehydrogenase (12) DNA construct (1808 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1356) selected/modified from the sequence of NADH-dependent butanol dehydrogenase (GenBank: YP_148778) of *Geobacillus kaustophilus*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1367-1788), and a PCR RE primer (1789-1808) at the 3' end.

[0380] Note, use of SEQ ID NOS. 166-179 in genetic transformation of a microbial host cell including (but not limited to) bacterial cells such as a cyanobacterium *Anabaena* PCC 7120 can create a designer cyanobacterium such as designer *Anabaena* with a designer reductive-acetyl-CoA biofuel-production pathway (numerically labeled as 83-90 and 07-12 in FIG. 13) for production of 1-butanol from hydrogen and carbon dioxide without requiring photosynthesis or sunlight. That is, the expression of SEQ ID NOS. 166-179 in a bacterium such as *Anabaena* PCC 7120 represents a designer organism with the designer hydrogenotrophic reductive-acetyl-CoA biofuel-production pathway (83-90 and 07-12 in FIG. 13) that can operate for anaerobic chemolithoautotrophic production of butanol from hydrogen and carbon dioxide even if it is in complete darkness.

[0381] SEQ ID NO: 180 presents example 180 of a designer hox-promoter-controlled Energy converting hydrogenase (Ech) (91) DNA construct (10538 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-10086) selected/modified from the sequence of Energy converting hydrogenase subunits (EchA, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q) (GenBank: ABC57807, and ABC57812-ABC57827) of *Methanospaera stadtmannae* DSM 3091, a 432-bp *Nostoc gor* terminator (10087-10518), and a PCR RE primer (10519-10538).

[0382] SEQ ID NO: 181 presents example 181 of a designer hox-promoter-controlled [NiFe]-hydrogenase MvhADG (95) DNA construct (3416 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-2964) selected/modified from the sequence of [NiFe]-hydrogenase MvhADG (GenBank: ADL59096, ADL59098, ADL59097) of *Methanothermobacter marburgensis*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (2965-3396), and a PCR RE primer (3397-3416).

[0383] SEQ ID NO: 182 presents example 182 of a designer hox-promoter-controlled Heterodisulfide reductases (HdrABC, HdrDE) (94) DNA construct (6695 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-6243) selected/modified from the

sequence of Heterodisulfide reductases (HdrABC, HdrDE) (GenBank: AET63985, AET63982, AET63983, AET64166, AET64165) of *Methanosaeta harundinacea*, a 432-bp *Nostoc gor* terminator (6244-6675), and a PCR RE primer (6676-6695).

[0384] SEQ ID NO: 183 presents example 183 of a designer hox-promoter-controlled Coenzyme F₄₂₀-reducing hydrogenase (Frh) (96) DNA construct (3407 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-2955) selected/modified from the sequence of Coenzyme F₄₂₀-reducing hydrogenase (FrhB1-3) (GenBank: YP_003357229, YP_003357467, YP_003357509) of *Methanocella paludicola* SANAe, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (2956-3387), and a PCR RE primer (3388-3407) at the 3' end.

[0385] Note, use of SEQ ID NOS. 180-183 in genetic transformation of a microbial host cell including (but not limited to) bacterial cells such as a cyanobacterium *Anabaena* PCC 7120 can confer an anaerobic chemolithoautotrophic hydrogen (H₂) utilization system [which, as shown in FIG. 12, comprises Energy converting hydrogenase (Ech) (91), [NiFe]-hydrogenase MvhADG (95), Coenzyme F₄₂₀-reducing hydrogenase (Frh) (96), and Coenzyme F₄₂₀-reducing hydrogenase (Frh) (96)] that can produce reducing power (Fd_{red}²⁻ and F₄₂₀H₂) from H₂ in support of the designer reductive-acetyl-CoA butanol-production pathway (83-90 and 07-12 in FIG. 13). Therefore, the expression of SEQ ID NOS. 180-183 along with SEQ ID NOS. 166-179 in a bacterium such as *Anabaena* PCC 7120 represents a designer organism (such as designer *Anabaena*) with a full designer reductive-acetyl-CoA biofuel-production pathway system (FIGS. 12 and 13) that can operate for anaerobic chemolithoautotrophic production of butanol from hydrogen and carbon dioxide without requiring photosynthesis or aerobic respiration. The net result in this example is the anaerobic chemolithoautotrophic production of butanol from hydrogen and carbon dioxide as shown in the process equation [21].

[0386] Also note, these designer genes (SEQ ID NOS. 166-183) are controlled by a designer hox anaerobic promoter. Therefore, under aerobic conditions such as in an open pond mass culture, the designer *Anabaena* in this example can quickly grow photoautotrophically using air carbon dioxide and water as the sources of carbon and electrons just like the wild-type parental strain. When the designer *Anabaena* cells cultures are grown and ready for use (as catalysts in this application), they can then be placed into an anaerobic reactor supplied with industrial CO₂ and H₂ gas for induction of the designer genes expression for anaerobic chemolithoautotrophic production of butanol (as shown in FIGS. 12 and 13) in dark.

[0387] SEQ ID NO: 184 presents example 184 of a designer hox-promoter-controlled Methyl-H4MPT: coenzyme M methyltransferase (MtrA-H) (92) DNA construct (5417 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-4965) selected/modified from the sequence of Methyl-H4MPT: coenzyme M methyltransferase (MtrA-H) (GenBank: ABC56714, ABC56713, YP_447360, YP_447354, YP_447359, YP_447355) of *Methanospaera stadmanae*, and mtrEF (AET65445, NC_009051) of *Methanosaeta harundinacea* and *Metha-*

noculleus marisnigri, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (4966-5397), and a PCR RE primer (5398-5417).

[0388] SEQ ID NO: 185 presents example 185 of a designer hox-promoter-controlled Methyl-coenzyme M reductase (Mcr) (93) DNA construct (5042 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-4590) selected/modified from the sequence of methylcoenzyme M reductase subunits A, B, C, G (GenBank: CAE48306, CAE48303, ABC56709, CAE48305) of *Methanospaera stadmanae*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (4591-5022), and a PCR RE primer (5023-5042).

[0389] Note, use of SEQ ID NOS. 184 and 185 along with SEQ ID NOS. 180-183 in genetic transformation of a microbial host cell including bacterial cells such as a cyanobacterium *Anabaena* PCC 7120 can confer a methanogenic hydrogenotrophic system which, as shown in FIG. 15, comprises Methyl-H4MPT: coenzyme M methyltransferase (MtrA-H) (92), Methyl-coenzyme M reductase (Mcr) (93), Energy converting hydrogenase (Ech) (91), [NiFe]-hydrogenase MvhADG (95), Coenzyme F₄₂₀-reducing hydrogenase (Frh) (96), Coenzyme F₄₂₀-reducing hydrogenase (Frh) (96). These enzymes along with a native ATPase 97 can produce ATP and reducing power (Fd_{red}²⁻ and F₄₂₀H₂) from H₂ in support of the designer reductive-acetyl-CoA methanogenic butanol-production pathways (FIGS. 16 and 17). Therefore, the expression of SEQ ID NOS. 180-185 along with SEQ ID NOS. 166-179 in a bacterium such as *Anabaena* PCC 7120 represents a designer organism (such as designer *Anabaena*) with a designer hydrogenotrophic reductive-acetyl-CoA methanogenic biofuel-production pathway system (FIGS. 15 and 17) that can operate for anaerobic production of both butanol and methane from hydrogen and carbon dioxide without requiring any photosynthesis. The net result in this example is the anaerobic chemolithoautotrophic production of butanol and methane from hydrogen and carbon dioxide as shown in the process equation [22].

[0390] SEQ ID NO: 186 presents example 186 of a designer hox-promoter-controlled Formate dehydrogenase (74) DNA construct (5450 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-4998) selected/modified from the sequence of formate dehydrogenase alpha and beta subunits (GenBank: AAB18330, AAB18329) of *Moorella thermoacetica*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (4999-5430), and a PCR RE primer (5431-5450).

[0391] SEQ ID NO: 187 presents example 187 of a designer hox-promoter-controlled 10-Formyl-H₄ folate synthetase (75) DNA construct (2324 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1872) selected/modified from the sequence of 10-formyltetrahydrofolate synthetase (GenBank: YP_428991) of *Moorella thermoacetica*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1873-2304), and a PCR RE primer (2305-2324).

[0392] SEQ ID NO: 188 presents example 188 of a designer hox-promoter-controlled 10-Methenyl-H₄ folate cyclohydrolyase (76) DNA construct (1487 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence

(193-1035) selected/modified from the sequence of methylenetetrahydrofolate cyclohydrolase (GenBank: YP_430368) of *Moorella thermoacetica* ATCC 39073, a 432-bp *Nostoc* gor terminator (1036-1467), and a PCR RE primer (1468-1487).

[0393] SEQ ID NO: 189 presents example 189 of a designer hox-promoter-controlled 10-Methylene- H_4 folate dehydrogenase (77) DNA construct (1487 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1035) selected/modified from the sequence of methylenetetrahydrofolate cyclohydrolase/5,10-methylenetetrahydrofolate dehydrogenase (GenBank: ABC19825) of *Moorella thermoacetica*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (1036-1467), and a PCR RE primer (1468-1487).

[0394] SEQ ID NO: 190 presents example 190 of a designer hox-promoter-controlled 10-Methylene- H_4 folate reductase (78) DNA construct (1565 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* (*Anabaena* PCC 7120) hox promoter (21-192), an enzyme-encoding sequence (193-1113) selected/modified from the sequence of methylenetetrahydrofolate reductase (GenBank: ABC19505) of *Moorella thermoacetica*, a 432-bp *Nostoc* gor terminator (1114-1545), and a PCR RE primer (1546-1565).

[0395] SEQ ID NO: 191 presents example 191 of a designer hox-promoter-controlled Methyl- H_4 folate: corrinoide iron-sulfur protein Methyltransferase (79) DNA construct (1442 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-690) selected/modified from the sequence of methyltetrahydrofolate:corrinoide/iron-sulfur protein methyltransferase (GenBank: YP_430174) of *Moorella thermoacetica*, a 432-bp *Nostoc* gor terminator (691-1122), and a PCR RE primer (1123-1442).

[0396] SEQ ID NO: 192 presents example 192 of a designer hox-promoter-controlled Corrinoid iron-sulfur protein (80) DNA construct (2942 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* hox promoter (21-192), an enzyme-encoding sequence (193-2490) selected/modified from the sequence of corrinoid iron-sulfur protein large and small subunits (GenBank: AEI90745, AEI90746) of *Clostridium autoethanogenum*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (2491-2922), and a PCR RE primer (2923-2942).

[0397] SEQ ID NO: 193 presents example 193 of a designer hox-promoter-controlled CO dehydrogenase/acetyl-CoA synthase (81) DNA construct (4859 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-4407) selected/modified from the sequence of carbon monoxide dehydrogenase alpha subunit alpha and beta subunits (GenBank: AAA23229, AAA23228) of *Moorella thermoacetica*, a 432-bp *Nostoc* gor terminator (4408-4839), and a PCR RE primer (4840-4859).

[0398] Note, use of SEQ ID NOS. 186-193 along with SEQ ID NOS. 174-179 in genetic transformation of a microbial host cell such as a cyanobacterium *Anabaena* PCC 7120 confers an ATP-requiring reductive-acetyl-CoA butanol-production pathway (74-81 and 07-12/42 in FIG. 14). Similarly, the expression of SEQ ID NOS. 186-193 and SEQ ID NOS. 180-185 along with SEQ ID NOS. 174-179 in a bacterium such as *Anabaena* PCC 7120 represents a designer organism (such as designer *Anabaena*) with a designer ATP-requiring

reductive-acetyl-CoA methanogenic biofuel-production pathway and a hydrogenotrophic methanogenesis-coupled ATP-generating system (FIGS. 15 and 16) that can operate for production of both butanol and methane from hydrogen and carbon dioxide. The net result in this example is the anaerobic chemolithotrophic production of both butanol and methane from hydrogen and carbon dioxide as shown in the process equation [22].

[0399] SEQ ID NO: 194 presents example 194 of a designer hox-promoter-controlled F_{420} synthesis enzymes (99) DNA construct (6428 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), enzymes-encoding sequence (193-4976) selected/modified from the sequence of lactaldehyde dehydrogenase CofA (GenBank: ADC46523,) of *Methanobrevibacter ruminantium*, 2-phospho-l-lactate guanylyltransferase (GenBank: ADL58588) of *Methanothermobacter Marburgensis*, 2-phospho-L-lactate transferase (GenBank: NP_987524) of *Methanococcus maripaludis*, coenzyme F420-0 gamma-glutamyl ligase (YP_001030766) of *Methanocorpusculum labreanum*, FO synthase subunits 1 and 2 (YP_003357513, YP_003357511) of *Methanocella paludicola*, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (4977-6408), and a PCR RE primer (6409-6428).

[0400] SEQ ID NO: 195 presents example 195 of a designer hox-promoter-controlled Pyridoxal phosphate-dependent L-tyrosine decarboxylase(mfnA for methanofuran synthesis) (100) DNA construct (1778 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzyme-encoding sequence (193-1326) selected/modified from the sequence of L-tyrosine decarboxylase (GenBank: YP_003355454) of *Methanocella paludicola*, a 432-bp *Nostoc* gor terminator (1327-1758), and a PCR RE primer (1759-1778).

[0401] SEQ ID NO: 196 presents example 196 of a designer hox-promoter-controlled Methanopterin synthesis enzymes (101) DNA construct (3215 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox promoter (21-192), an enzymes-encoding sequence (193-2763) selected/modified from the sequence of GTP cyclohydrolase (GenBank: YP_447347) of *Methanosphaera stadtmanae* DSM 3091, cyclic phosphodiesterase MptB (AB035741) of *Methanococcus maripaludis* C5, beta-ribofuranosylaminobenzene 5'-phosphate synthase (YP_003356610) of *Methanocella paludicola* SANAE, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (2764-3195), and a PCR RE primer (3195-3215).

[0402] SEQ ID NO: 197 presents example 197 of a designer hox-promoter-controlled Coenzyme M synthesis enzymes (102) DNA construct (4226 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Nostoc* sp. strain PCC 7120 (*Anabaena* PCC 7120) hox promoter (21-192), an enzymes-encoding sequence (193-3774) selected/modified from the sequence of phosphosulfolactate synthase, 2-phosphosulfolactate phosphatase and sulfolactate dehydrogenase (GenBank: ADL57861, YP_003850451, ADL59162) of *Methanothermobacter marburgensis*, and sulfopyruvate decarboxylase (YP_003357048) of *Methanocella paludicola* SANAE, a 432-bp *Nostoc* sp. strain PCC 7120 gor terminator (3775-4026), and a PCR RE primer (4027-4226).

[0403] SEQ ID NO: 198 presents example 198 of a designer hox-promoter-controlled Coenzyme B synthesis enzymes (103) DNA construct (5198 bp) that includes a PCR FD primer (sequence 1-20), a 172-bp *Anabaena* PCC 7120 hox

promoter (21-192), an enzymes-encoding sequence (193-4746) selected/modified from the sequence of isopropylmalate synthase, isopropylmalate dehydrogenase (GenBank: AAM01606, NP_614498) of *Methanopyrus kandleri*, isopropylmalate isomerase large and small subunits (ADP98363, ADP98362) of *Marinobacter adhaerens*, a 432-bp *Nostoc gor* terminator (4747-5178), and a PCR RE primer (5179-5198).

[0404] Note, the expression of SEQ ID NOS. 194-198 in a microbial host cell such as cyanobacterium *Anabaena* PCC 7120 provides the ability of synthesizing some of the cofactors such as F₄₂₀, methanofuran, methanopterin, Coenzyme M, and Coenzyme B that are needed for the designer hydrogenotrophic reductive-acetyl-CoA biofuel-production pathways (of FIGS. 13, 14, 16 and 17) to properly operate. Depending on the genetic backgrounds of various host cells such as cyanobacteria, many of them may or may not possess some of these enzymes to synthesize this type of special cofactors. Therefore, in one of the various embodiments, it is a preferred practice to express this type of designer cofactor-synthesis enzymes (e.g., SEQ ID NOS. 194-198) along with the hydrogenotrophic designer reductive-acetyl-CoA biofuel-production pathway genes (e.g., SEQ ID NOS. 166-193) as shown in these examples.

[0405] Note, many of the hydrogenotrophic bacteria and methanogens such as *Methanocella paludicola* SANAE naturally possess certain hydrogenotrophic and/or reductive acetyl-CoA pathway(s) and the ability of synthesizing the associated cofactors including F420, methanofuran, methanopterin, Coenzyme M, and Coenzyme B. Therefore, in one of the various embodiments, it is also a preferred practice to express certain designer genes of biofuel-production-pathways (FIGS. 1, 4, 5, 6, 7, 8, 10, 13, and 14) such as SEQ ID NOS. 174-179 in a hydrogenotrophic and/or methanogenic host cell for chemolithotrophic production of advanced biofuels such as 1-butanol from hydrogen (H₂) and carbon dioxide (CO₂). According to one of the various embodiments, a hydrogenotrophic and/or methanogenic host organism for this specific application is selected from the group consisting of: *Methanocella paludicola* SANAE, *Acinetobacter baumannii* ABNIH3, *Acinetobacter baumannii* ABNIH4, *Acinetobacter* sp. DR1, *Agrobacterium* sp. H13-3; *Agrobacterium vitis* S4, *Alcaligenes* sp., *Allochromatium vinosum* DSM 180, *Amycolatopsis mediterranei* S699, *Anoxybacillus flavithermus* WK1, *Aquifex aeolicus* VF5, *Archaeoglobus fulgidus* DSM 4304, *Archaeoglobus veneficus* SNP6, *Azospirillum* sp. B510, *Burkholderia cenocepacia* HI2424, *Caldicellulosiruptor bescii* DSM 6725, *Carboxydotherrmus hydrogenoforans*, *Centipeda periodontii* DSM 2778, *Clostridium autoethanogenum*, *Clostridium ragsdalei*, *Clostridium sticklandii* DSM 519, *Clostridium sticklandii*, *Corynebacterium glutamicum*, *Cupriavidus metallidurans* CH34, *Cupriavidus necator* N-1, *Desulfobacca acetoxidans* DSM 11109, *Exiguobacterium* sp. AT1b, *Ferrimonas balearica* DSM 9799, *Ferroglobus placidus* DSM 10642, *Geobacillus kaustophilus*

HTA426, *Helicobacter bilis* ATCC 43879, *Herbaspirillum seropedicae* SmR1, *Hydrogenobacter thermophilus* TK-6, *Hydrogenovibrio marinus*, *Klebsiella variicola* At-22, *Methanobacterium* sp. SWAN-1, *Methanobrevibacter ruminantium* M1, *Methanocaldococcus fervens* AG86, *Methanocaldococcus infernus* ME, *Methanocaldococcus jannaschii*, *Methanocaldococcus* sp. FS406-22, *Methanocaldococcus vulcanius* M7, *Methanococcus aeolicus* Nankai-3, *Methanococcus maripaludis* C6, *Methanococcus maripaludis* S2, *Methanococcus voltae* A3, *Methanocorpusculum labreanum* Z, *Methanoculleus marisnigri* JR1, *Methanohalophilus mahii* DSM 5219, *Methanolinea tarda* NOBI-1, *Methanoplanus petrolearius* DSM 11571, *Methanoplanus petrolearius*, *Methanopyrus kandleri* AV19, *Methanoregula boonei* 6A8, *Methanosaeta harundinacea* 6Ac, *Methanosalsum zhilinae* DSM 4017, *Methanosarcina acetivorans* C2A, *Methanosarcina barkeri* str. Fusaro, *Methanosarcina mazei* Go1, *Methanosphaera stadtmanae*, *Methanospirillum hungatei* JF-1, *Methanothermobacter marburgensis* str. Marburg, *Methanothermobacter marburgensis*, *Methanothermobacter thermautotrophicus*, *Methanothermococcus okinawensis* IH1, *Methanothermus fervidus* DSM 2088, *Methylobacillus flagellates*, *Methylobacterium organophilum*, *Methylococcus capsulatus*, *Methylomicrobium kenyense*, *Methylomonas methanica* MC09, *Methylomonas* sp. LW13, *Methylomonas* sp. LW2, *Methylosinus trichosporium* OB3b, *Methylothermobacter mobilis* JLW8, *Methylothermobacter versatilis* 301, *Methylovorus glucosetrophus* SIP3-4, *Moorella thermoacetica* ATCC 39073, *Moorella thermoacetica*, *Oligotropha carboxidovorans* OM5, *Paenibacillus terse* HPL-003, *Pelotomaculum thermopropionicum* SI, *Planctomyces brasiliensis* DSM 5305, *Pyrococcus furiosus* DSM 3638, *Pyrococcus horikoshii* OT3, *Pyrococcus yayanosii* CH1, *Ralstonia eutropha* H16, *Rubrivivax* sp., *Selenomonas noxia* ATCC 43541, *Shewanella baltica* BA175, *Stenotrophomonas* sp. SKA14, *Synechococcus* sp. JA-2-3B' a(2-13), *Synechococcus* sp. JA-3-3Ab, *Thermococcus gammatolerans* EJ3, *Thermococcus kodakarensis* KOD1, *Thermococcus onnurineus* NA1, *Thermococcus* sp. 4557, *Thermodesulfatator indicus* DSM 15286, *Thermofilum pendens* Hrk 5, *Thermotoga lettingae* TMO, *Thermotoga petrophila* RKU-1, *Thiocapsa roseopersicina*, *Thiomonas intermedia* K12, *Xanthobacter autotrophicus*, *Yersinia pestis* Antigua, and combinations thereof.

[0406] While the present invention has been illustrated by description of several embodiments and while the illustrative embodiments have been described in considerable detail, it is not the intention of the applicant to restrict or in any way limit the scope of the appended claims to such detail. Additional advantages and modifications will readily appear to those skilled in the art. The invention in its broader aspects is therefore not limited to the specific details, representative apparatus and methods, and illustrative examples shown and described. Accordingly, departures may be made from such details without departing from the spirit or scope of applicant's general inventive concept.

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designer Butyraldehyde-Dehydrogenase DNA construct (2067 bp)

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<400> SEQUENCE: 4

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gcgcatgaaa atgcattcgc ttccatagga cgctgcattg tggettgaag gttcaaggga   180
agggttcaaa cgaccccgcc gtacgaactt ttgtcggggg gcgctcccgg ccccgggctc   240
ttgtgcgcgc attagggtct cgggtcgcaa gcaagacgat acctcgagca tatggccgcc   300
gtcattgcca agtcctccgt ctccgcggcc gtggtctgcc cggcccgctc cagcgtgccc   360
cccattggcc cgctgaagcc cgccgtcaag gctgcccccg tggctgcccc ggctcaggcc   420
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gttttagatg atttagaagc agacaacaat gtgtatgcag ttatagttac tgggtctggt   600
gagaaatctt ttgttgctgg agcagatatt tcagaaatga aagatcttaa tgaagaacaa   660
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ccagttatcg cagctatata aggatttctt cttggtggtg gatgtgaact tgctatgtca   780
tgtgacataa gaatagcttc agttaaagct aaatttggtc aaccagaagc aggacttggg   840
ataactccag gatttgggtg aactcaaaag ttagcaagaa tagttggacc aggaaaagct   900
aaagaattaa ttatactctg tgaccttata aatgcagaag aagcttatag aataggctta   960
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cggtagctta gctccccgtt tctgctgctg cagtcttttt caacacgtaa aaagcggagg  1380
agttttgcaa ttttgttggg tgtaacgatc ctccgttgat tttggcctct ttctccatgg  1440
gcgggctggg cgtatttgaa gcggttctct cttctgccgt ta                    1482

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<210> SEQ ID NO 5

<211> LENGTH: 1367

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 5, Example 5:
designer 3-Hydroxybutyryl-CoA-Dehydrogenase DNA construct
(1367 bp)

<400> SEQUENCE: 5

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt    60
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ccgtcattgc caagtctccc gtctccggcg ccgtggctcg cccggcccgc tccagcgtgc   180
gccccatggc cgcgctgaag cccgcgctca aggctgcccc cgtggctgcc cgggctcagg   240
ccaaccagat gaaaaagatt tttgtacttg gagcaggaac tatgggtgct ggtatcgctc   300
aagcattcgc tcaaaaaggg tgtgaggtaa ttgtaagaga cataaaggaa gaatttgttg   360

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acagaggaat agctggaatc actaaaggat tagaaaagca agttgctaaa ggaaaaatgt 420
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ctgctgactg tgatttagta gttgaagctg caatcgaaaa catgaaaatt aagaaggaaa 540
tctttgctga gtttagatgga atttgtaagc cagaagcgat tttagcttca aacacttcat 600
ctttatcaat tactgaagtt gcttcagcta caaagagacc tgataaagtt atcggaatgc 660
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aagttgcaga agctccagga ttcggtgtaa acggaatctt aatccaatg attaacgaag 840
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atggtgctaa ccatccaatg ggacctttag ctttaggaga tcttattgga ttagatgttt 960
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ggaggagttt tgcaattttg ttggtgtaa cgatcctcgg ttgattttgg cctctttctc 1320
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<210> SEQ ID NO 6

<211> LENGTH: 1721

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 6, Example 6:
designer Thiolase DNA construct (1721 bp)

<400> SEQUENCE: 6

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgctcgact tgaagggtt 60
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ccgtcattgc caagtctccc gtctcccgcg ccgtggctcg cccggcccgc tccagcgtgc 180
gccccatggc cgcctgtaag cccgcctca aggctgcccc cgtggctgcc ccgctcagg 240
ccaaccagat gggcaaaaga agtagtttta gctgtgcatg tegtacagcc atcggaacaa 300
tgggtggate tcttagcaca attcctgcag tagatttagg tgctatcgtt atcaaaagag 360
ctcttaaccg cgcaggtggt aaacctgaag atgttgatca cgtatacatg ggatgcgtta 420
ttcaggcagg acaggacag aacgttgctc gtcaggcttc tatcaaggct ggtcttctcg 480
tagaagtacc tgcagttaca actaacgttg tatgtggttc aggtcttaac tgtgttaacc 540
aggcagctca gatgatcatg gctggagatg ctgatatcgt tgttccgggt ggtatggaaa 600
acatgtcact tgcaccattt gcaacttcta atggccgtta cggatatcgt atgatgtggc 660
caagccagag ccagggtggt cttgtagaca ctatggttaa ggatgctctt tgggatgctt 720
tcaatgatta tcaatgatc cagacagcag acaacatctg cacagagtgg ggtcttacac 780
gtgaagagct cgatgagttt gcagctaaga gccagaacaa ggcttctgca gcaatcgaag 840
ctggcgatt caaggatgag atcgttctcg tagagatcaa gaagaagaaa gagacagtta 900
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gtcctatcaa caaggatgga ttcgttacag ctggtaacgc ttcaggtatc aacgacggtg 1020
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ctacattcgt agctggagca cttgctgggt ttcgtcctga agttatgggt atcggctctg 1140
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tcgacgtcca caagcagctc aatcctaacg gtgggtctat cgctcttggg caccagttg 1320
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cgggctgggc gtatttgaag cggttctctc ttctgccgtt a 1721

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<210> SEQ ID NO 7

<211> LENGTH: 4211

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 7, Example 7:
designer Pyruvate-Ferredoxin-Oxidoreductase DNA construct
(4211 bp)

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<400> SEQUENCE: 7

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gacccccgcg gacttgaag ggttcaaacg acccccgctg acgaactttt gtcggggggc 180
gtccccggct cgagcatatg gccgcgctca ttgccaagtc ctccgtctcc gcgccgctgg 240
ctcgccccgc ccgctccagc gtgcgcccc a tggccgctgct gaagccccgc gtcaggctg 300
cccccgctggc tgccccggct caggccaacc agatggcgca gaggtgcaag gagccccctg 360
acggaacgac agccaagcag cacgtggcct acttcatgag cgacagcgcg ttcattctcc 420
ccatcacgcc cagctcggtc atgtccgagg tcgcccacga gtggtccatg aacggccgca 480
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gcgccctgca cggcgcgctc agcgaggag cgctggcgac gacgttcacg agcagccagg 600
gctgctgct catgatcccc aacatgtaca agatcgccgg cgagctcctg cctgctgca 660
tgacatcgc cccccgacc gtcgcccacc aggcctctc tatcttcggc gaccacacgg 720
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cgatgatgga gcaccagatg gtccgcggga tgaaggagag ccagaagaac cagaagctgg	3660
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ttctgccgtt a	4211

<210> SEQ ID NO 8

<211> LENGTH: 2021

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 8, Example 8:
designer Pyruvate-Kinase DNA construct (2021 bp)

<400> SEQUENCE: 8

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gccccatggc cgcgctgaag cccgcgctca aggctgcccc cgtggctgcc ccggctcagg	240
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cattggccat tgctttggac accaagggtc cagaaatcag aactggtacc accaccaacg	540
atgttgacta cccaatccca ccaaaccacg aaatgatctt caccaccgat gacaagtacg	600
ctaaggcttg tgacgacaag atcatgtacg ttgactacaa gaacatcacc aaggctcatct	660
ccgctggtag aatcatctac gttgatgatg gtgttttctc tttccaagtt ttggaagtgc	720
ttgacgacaa gactttgaag gtcaaggctt tgaacgccgg taagatctgt tcccacaagg	780
gtgtcaactt accaggtagc gatgtcgatt tgccagcttt gtctgaaaag gacaagggaag	840
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ccaacgatgt ttgaccatc agagaagtct tgggtgaaca aggtaaggac gtcaagatca	960
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acgggtgtat ggttgccaga ggtgacttgg gtattgaaat cccagcccca gaagtcttgg	1080
ctgtccaaaa gaaattgatt gctaagtcta acttgctggg taagccagtt atctgtgcta	1140
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ctatcgctta cttgccaaac tacgatgaca tgagaaactg tactccaaag ccaacctcca 1380
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ggtagcttag ctccccgttt cgtgctgac agtctttttc aacacgtaaa aagcggagga 1920
gttttgcaat tttgttggtt gtaacgatcc tccgttgatt ttggcctctt tctccatggg 1980
cgggctgggc gtatttgaag cggttctctc ttctgccgtt a 2021

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<210> SEQ ID NO 9

<211> LENGTH: 1815

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 9, Example 9:
designer Enolase DNA construct (1815 bp)

<400> SEQUENCE: 9

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agggttcaaa cgacccccgc gtacgaactt ttgtcggggg gcgctcccgg ccccgggctc 240
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gatcagtcct tttcaacacg taaaagcgg aggagtcttg caattttgtt ggttgaacg 1740
atcctcgtt gattttggcc tctttctcca tgggcgggct gggcgtattt gaagcgttc 1800
tctctctgc cgta 1815

```

<210> SEQ ID NO 10

<211> LENGTH: 2349

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 10, Example 10: designer Phosphoglycerate-Mutase DNA construct (2349 bp)

<400> SEQUENCE: 10

```

agaaaatctg gcaccacacc tatatggtag ggtgcgagtg accccgcgcg acttgagct 60
cgatggcccc gggttgtttg gggcgtccgc ctctcgcgct attctgagct ggagaccgag 120
gcgatgaaa atgcattcgc ttccatagga cgctgcattg tggcttgaag gttcaaggga 180
agggttcaaa cgacccccgc gtacgaactt ttgtcggggg gcgctcccgg ccccggtctc 240
ttgtgcgcgc attagggctt cgggtcgcaa gcaagacgat acctcgagca tatggccgcc 300
gtcattgcca agtcctccgt ctcccgccgc gtggctcgc cggcccctc cagcgtgccc 360
ccccatggcg cgctgaagcc cgcctcaag gctgcccccg tggctgcccc ggetcaggcc 420
aaccagatgg cgcacgacta caagetgaag gcccccggcg cgattcctgc gcccgagggc 480
ccgctgctgg tctgcattct ggacggcttc ggcgagaac agtacaagga tgagttcaac 540
gccgtgcacg tggctaaagc gccactgtg gacgcgctgc gcgctgtgcc ccatcgcttc 600
cgttccatca aggcgcacgg aaaggctgtg ggctgcccga gcgatgccga catgggcaac 660
agcaggtgg ggcacaacgc cctgggctcg ggcaggtgg tggaccaagg cgcgcgctg 720
gtggacctg cgctggagac cggccgtatg ttctcggacc cggctggaa gctcatcagc 780
gaggccttc cctcccacac cgtccacttc atcggcctgc tgtccgacgg cggcgtgca 840
tcgcgcgccg atcagctgca cggctgcctg cgcggcgccg tggagcggcg cccaagcgc 900
gtgcgcgtgc acatcctgac tgacggccgc gacgtgcccg acggcagcag catccggctc 960
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gcctcgggcg gggcccgcat gcaggtcacc atggaccgct acgaggcggg ctggagcatg 1080
gtgaagcgcg gctgggacgc gcacgtgctg ggcaaggcgc cccactactt caaggacgcc 1140
aagaccggcg tcaccacct gcgcggtccc gaggacgcgc cggtgtctga ccagtacgtg 1200
gccccctttg tgattgtgga cgaggcggac aagccggtgg gcaccattga ggaagcgcgac 1260

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gcggtggtgc tgttcaactt ccgcgcgac cgcattggtg agatcagcaa ggccttcgag 1320
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ggcatgatgc agtacagcgg cgacctgaag ctgcccgcga acttctggtt gccgcccgcc 1440
ctgattgagc acgtgtcggg cgagtacctg tgcaagaacg ggctgagcac ctctgcctgc 1500
tccgagactc agaagtccgg gcacgtgacg ttcttctgga acggcaaccg ctccgctac 1560
ctggacgcca agcaggagca gtacctggag atcccgtcgg acaagatcga gttcaacaag 1620
gctccggaca tgaaggcggc cgagatcacc gcccccgcga ttgagggcct caagagcggc 1680
aagtacaagg tgggtgcgat caactacgcc aaccggaca tggctcgcca caccggcgac 1740
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gtggtggaca gcctgaacgg ccgctggatc gtcacgtccg accacggcaa cgccgacgac 1860
atgggtcagc gcgacaagaa gggcaagccc ctgctgggcg aggacggcaa gccgctgccc 1920
ctgaccagcc acacgtggc gccctgcccg ttcttcatcg gggcaaggg cctgcccggc 1980
ggcgtggtgc tgcgagcaga cctgcccggc gccgggctgg ccaacgtggc cgccaccacc 2040
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tctagataaa tggaggcgct cgttgatctg agccttgcgc cctgacgaac ggcggtggat 2160
ggaagatact gctctcaagt gctgaagcgg tagcttagct ccccgtttcg tgctgatcag 2220
tctttttcaa cacgtaaaaa gcggaggagt tttgcaattt tgttggtgtt aacgatcctc 2280
cgttgatatt ggctctcttc tccatgggcg ggctgggctg atttgaagcg gttctctctt 2340
ctgcccgtta 2349

```

<210> SEQ ID NO 11

<211> LENGTH: 1908

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 11, Example 11: designer Phosphoglycerate-Kinase DNA construct (1908 bp)

<400> SEQUENCE: 11

```

agaaaatctg gcaccacacc tatatggtag ggtgagatg accccgcgcg acttgagct 60
cgatggcccc ggggtgtttg gggcgtcccg ctctcgcgct attctgagct ggagaccgag 120
gcgcatgaaa atgcattcgc tccatagga cgctgcattg tggcttgaag gttcaaggga 180
agggttcaaa cgaccccgcg gtacgaactt ttgtcggggg gcgctcccgg ccccgggctc 240
ttgtgcgcgc attagggcct cgggtcgcaa gcaagacgat acatggccct ctctatgaag 300
atgcgcgcca acgcgcgcggt gtcgggtcgc cgcgtcgcg ctgtggcccc ccgctggtg 360
cccttctcgt cggcctccag ctccgtgctg cgctctggct tcgctgctgag gtgtctgtgg 420
acatccgccc cgtgggcccg tctcgcaccc gtcgtcaggg cggtaagaa gtcggttggc 480
gacctgcaca aggctgacct ggagggcaag cgcgtgttcg tccgcgcgga cctgaacgtg 540
cctcttgaca aggccaccct ggccatcacc gacgacacc gcattcgcgc gcccgcccc 600
accctgaagt acctgctgga caacgggtgct aaggtcctgc tgacctgca cctgggtcgc 660
ccgaaggggc gtcccagga caagtaccgc ctgacccccg tggtgcccgc cctgctggag 720
ctgctgggca agcccgtgac caaggtgat gactgcatcg gcccagaggt ggagaaggcg 780
gtggcgccca tgaagaacgg cgagctgctg ctgctggaga actgccgctt ctacaaggag 840

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gaggagaaga acgagcccga gttcgccaag aagctggccg ccaacgccga cctgtacgtg   900
aacgacgcgt tcggcactgc ccaccgcgcc cacgcctcca ccgaggggtg gaccaagttc   960
ctgaagccct ccgtggccgg ctctctgctg cagaaggagc tggactacct tgatggcgcc  1020
gtgtccaacc ccaagcgccc ctctgtggcc attgtgggcg gctccaaggt gtcctccaag  1080
atcaccgtca ttgaggcgct gatggagaag tgcgacaaga tcatcatcgg cggtggeatg  1140
atcttcacct tctacaaggc ccgcgcgctg aaggtgggct cctcgctggt tgaggacgac  1200
aagatcgagc tggccaagaa gctggaggag atggccaagg ccaaggggtg gcagctgctg  1260
ctgcccaccg acgtggtggt ggccgacaag ttcgacgcc aacccaacac ccagaccgtg  1320
cccatcaccg ccatccccga tggctggatg ggtctggaca ttggcccga ctcctcaag  1380
accttcaacg acgccctggc cgacgccaa accgtttgtg ggaacggccc catgggtgtg  1440
ttcgagtctt ccaagtctg ccaacgcacc gtgtcgatcg ccaacacct ggccggcctg  1500
acgcccgaag gctgcatcac catcattggt ggcggtgact ccgtggtgct cgtcgagcag  1560
gccggcgttg ccgagaagat gagccacatc tccaccggcg gcggtgcctc cctggagctg  1620
ctggagggca aggtcctgcc cggcgtggcc gccctggacg agaagtaaat ggaggcgtc  1680
gttgatctga gccttgcccc ctgacgaacg gcggtggatg gaagatactg ctctcaagtg  1740
ctgaagcggg agcttagctc ccctttctgt gctgatcagt ctttttcaac acgtaaaaag  1800
cggaggagtt ttgcaatctt gttggttgta acgatcctcc gttgatcttg gcctctttct  1860
ccatggggcg gctgggcgta tttgaagcgg ttctctcttc tgccgtta   1908

```

<210> SEQ ID NO 12

<211> LENGTH: 1677

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 12, Example
12: designer NAD-dependent Glyceraldehyde-3-Phosphate-
Dehydrogenase DNA construct (1677 bp)

```

<400> SEQUENCE: 12

```

agaaaatctg gcaccacacc tatatggtag ggtgcgagtg accccgcgcg acttgagct   60
cgatggcccc gggttgtttg gggcgtccgc ctctcgcgct attctgagct ggagaccgag  120
gcgcatgaaa atgcattcgc ttccatagga cgctgcattg tggcttgaag gttcaaggga  180
agggttcaaa cgaccccgcc gtacgaactt ttgtcggggg gcgctcccgg ccccgggctc  240
ttgtgcgcgc attagggctt cgggtcgcaa gcaagacgat acatggccgc cgtcattgcc  300
aagtcctccg tctccggcgc cgtggctcgc ccggcccgcct ccagcgtgcg ccccatggcc  360
gcgctgaagc ccgccgtaaa ggctgccccg gtggctgccc cggctcaggc caaccagatg  420
gctcccatca agatcggcat caatggtttt ggctgtattg gccgcctcgt gtggcgtgcc  480
actcttaacc gtgacgatgt cgaggctcgc gccatcaatg atccattcat tgatgtgcca  540
tacatggtct acatggccaa gtatgactcg gtccacggca acctgaccca cgacgttcag  600
caaggcgagc gcaagctgat ggtcaatggc aagtcaatca ccatcttcgg caagatggat  660
gccaaaggaga tcccatggaa ggaggccggc gcgaccttcg tcgttgagtc gactgggtgtg  720
ttcaccaccc tggaggggcg cagctctcac ctggtcggcg gtgctgagac cgtcgtcatc  780
tccgcccctt caaacgatgc ccccatgttc gtcatgggtg tcaacgagga gggctacaag  840

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ccagacatga aagtgggtgtc caacgcgtct tgcaccacca actgcctggg cccctggcc 900
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accgccacccc agaagaccgt cgacgggccc tccaagaagg actggcgccg cgggcgccc 1020
atcctggaca acatcatccc ctccggcact ggtgcccga aggcctcgg caaggtgctg 1080
cctgccctga acggcaagct caccggcatg gccttccgctg tgcccacccc cgatgtctcg 1140
gtcgtcgate tgaccgtgct cctggagaag ggtgctcgt acgacgccat caaggccgag 1200
atcaagcgcg cgagcgagaa cgagctcaag ggcatcctgg cctacaccga ggatgccgtg 1260
gtctccaccg acttcatcgg caacaagcac agctccatct tcgacgccga ggcggcacc 1320
gccctcaacg acaactttgt caagctggtc tctgtgtacg acaacgagtg gggctactcc 1380
aacctgtgctg tcgacctgat cgcgcacatg gccaaggcca aggcggccag ccactaaatg 1440
gagggcgtcgt ttgatctgag ccttgcccc tgacgaaagg cgggtggatgg aagatactgc 1500
tctcaagtgc tgaagcggta gcttagctcc ccgtttcgtg ctgatcagtc tttttcaaca 1560
cgtaaaaagc ggaggagttt tgcaattttg ttggtttaa cgatcctccg ttgattttgg 1620
cctctttctc catggggcgg ctgggcgtat ttgaagcggg tctctctctt gccgtta 1677

```

<210> SEQ ID NO 13

<211> LENGTH: 2351

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 13, Example
13: designer HydA1-promoter-linked Phosphoglycerate-Mutase DNA
construct (2351 bp)

```

<400> SEQUENCE: 13

```

agaaaatctg gcaccacacc gagctgtcat gcgttgttcc gttatgtgtc gtcaaacgcc 60
ttcgagcgtc gcccggaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gcccggctgac tggcacaagg gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccc ctgcattccc gccggattgg gaaatcgcga tggcgcgcga taggcaagct 240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc 300
aaatggcccgc cgtcattgcc aagtcctccg tctccgccc cgtggctcgc cgggcccgtc 360
ccagcgtgctg ccccatggcc gcgctgaagc ccgcccgtca ggctgcccc gtggtgccc 420
cggctcaggc caaccagatg gcgcacgact acaagctgaa ggcccacccg gcgattcctg 480
cgcccagggg cccgctgctg gtctgcattc tggacggctt cggcgagaac gagtacaagg 540
atgagttcaa cgcctgacac gtggctaaga cgcacctgt ggacgcctg cgcgctgtgc 600
cccatecgtt ccgttccatc aaggcgcacg gaaaggctgt gggcctgccc agcgtatgcc 660
acatgggcaa cagcgagggtg gggcacaacg ccctgggctc gggccagggt gtggaccaag 720
gcgcgcgcct ggtggacctg gcgctggaga ccggccgtat gttctcggac cccggctgga 780
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gcgccaagcg cgtgcgcgctg cacatcctga ctgacggccg cgacgtgccc gacggcagca 960
gcatccgggtt cgtggaggag ctggaggcgg tgctggcgga gctgcgcggc aagggtgctg 1020
acatcgccat ccctcgggc ggcggcccga tgcaggtcac catggaccgc tacgaggcgg 1080
actggagcat ggtgaagcgc ggctgggacg cgcacgtgct gggcaaggcg cccactact 1140

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tcaaggacgc caagaccgcg gtcaccaccc tgcgcggctc cgaggacgcg ccggtgtctg 1200
accagtagct ggcccccttt gtgattgtgg acgaggcgga caagccggtg ggcaccattg 1260
aggacggcga cgcggtggtg ctgttcaact tccgcgcgga ccgcatggtg gagatcagca 1320
aggccttcga gtacaggac ggcttcaccg cctttgagcg cgagcgcttc cccaagggcc 1380
tgcgcttcgt gggcatgatg cagtagcagc gcgacctgaa gctgcccgcc aacttcctgg 1440
tgcgcgcgcc cctgattgag cacgtgtcgg gcgagtacct gtgcaagaac gggctgagca 1500
ccttcgcctg ctccgagact cagaagtctg ggcacgtgac gttcttctgg aacggcaacc 1560
gctccggcta cctggacgcc aagcaggagc agtacctgga gatcccgtcg gacaagatcg 1620
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tcaagagcgg caagtacaag gtggtgcgca tcaactacgc caaccggac atggtcggcc 1740
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agctgctgga ggtggtggac agcctgaacg gccgctggat cgtcacgtcc gaccacggca 1860
acgccgacga catggtgcag cgcgacaaga agggcaagcc cctgctgggc gaggacggca 1920
agccgctgcc cctgaccagc cacacgctgg cgcccgtgcc gttcttcacg ggcggcaagg 1980
gectgcggga cggcgtggtg ctgcgcgacg acctgccgga cgccgggctg gccaacgtgg 2040
ccgccaccac cttcaacctg ctgggcttcg aggcgcccgg catctacaag cccagcatgg 2100
tcaaggcgta aatggaggcg ctcgttgatc tgagccttgc cccctgacga acggcggtgg 2160
atggaagata ctgctctcaa gtgctgaagc ggtagcttag ctccccgttt cgtgctgatc 2220
agtctttttc aacacgtaaa aagcggagga gttttgcaat tttgttggtt gtaacgatcc 2280
tccggtgatt ttggcctctt tctccatggg cgggctgggc gtatttgaag cggttctctc 2340
ttctgcggtt a 2351

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<210> SEQ ID NO 14

<211> LENGTH: 1796

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 14, Example
14: designer HydA1-promoter-linked Enolase DNA construct
(1796 bp)

<400> SEQUENCE: 14

```

agaaaatctg gcaccacacc gagctgtcat gcggtgttcc gttatgtgtc gtcaaacgcc 60
ttcgagcgct gcccgaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gcgggctgac tggcacaaggc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccc ctgcattccc gccggattgg gaaatcgcga tggctcgcga taggcaagct 240
cgaaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtcctccg tctccgcgcc cgtggctcgc ccggcccgcct 360
ccagcgtgcg ccccatggcc gcgctgaagc ccgcccgtcaa ggctgcccccc gtggctgccc 420
cggctcaggc caaccagggt accaaggctg ttgagaacat caacgctatt attgcccccg 480
ccctgaaggg catggacccc gtcaagcagg cggagattga ccagaagatg aaggacctgg 540
acggcactga caacaagggc aagctgggtg ccaacgccat cctggccgctc tccatggccg 600
tgtgcaaggc cgggtgccct gagaagggcg tgcccctgta caagcacatt gcggacctgg 660

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ccggcaacag caagctgac ctagccgtgc cctcgttcaa catcatcaac ggccggcagcc 720
acgccggcaa cgccctggct atgcaggagt tcatgatcct gcccggtggc gectcgagct 780
tctctgaggg catgcgcatg ggctgcgagg tgtaccacgc cctgaagggc ctgatcaagg 840
ccaagtacgg ccaggacgcc tgcaacgtgg gtgatgaggg tggcttcgcc cccaacatcg 900
gtccaacga tgagggcctg aacttggtga acgaggccat cgagaaggcc ggtacacccg 960
gcaaggtgaa gatcggcatg gacgtggcct cgtcggagtt ctacaccgag gacggcatgt 1020
acgacctgga cttcaagaac cagcccaacg atggctcgca gaagaagacc aaggagcaga 1080
tgctggagct gtacaacgag ttctgcaaga agtaccgggt catctccatc gaggaccct 1140
tcgagcagga cgactgggag ccctgcgcca agctgaccac cgagaacatc tgccaggtgg 1200
tcggcgacga catcctgggtg accaaccctg tgccgctgaa gaaggccatc gacgccaagg 1260
ccgtcaacgc tctgctgctc aaggtaacc agatcggtag cattaccgag tccattgagg 1320
ccgtgcgcat ggccaaggag gccggctggg gtgtcatgac cagccaccgc tcgggtgaga 1380
ctgaggactc tttcatcgcc gacctggcgg tgggcctggc ctccggccag atcaagaccg 1440
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agctggggca gaacgctgtg tacgctggcg agagctggcg ccacatcggc tggtaaagg 1560
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ctcaagtget gaagcggtag cttagctccc cgtttcgtgc tgatcagtct ttttaacac 1680
gtaaaaagcg gaggagtttt gcaattttgt tggttgtaac gatcctccgt tgattttggc 1740
ctctttctcc atgggggggc tgggcgtatt tgaagcggtt ctctctctg ccgtta 1796

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<210> SEQ ID NO 15

<211> LENGTH: 1832

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 15, Example
15: designer HydA1-promoter-linked Pyruvate-Kinase DNA construct
(1832 bp)

<400> SEQUENCE: 15

```

agaaaatctg gcaccacacc gagctgtcat gcggtgttcc gttatgtgtc gtcaaacgcc 60
ttcgagcgtc gcccggaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gcccggctgac tggtaaacgc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggctcgcga taggcaagct 240
cgaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtccctcg tctccggcgc cgtggctcgc ccggcccgtc 360
ccagcgtgag ccccatggcc gcgctgaagc ccgcccgtcaa ggctgcccc gtggctgccc 420
cggtcagcgc caaccagatg tgcgagatgc tggacgcggg cgtggtgggc tgcgcgctgg 480
acctgacgtg gggcccgtcg gagttccacc gcaagtcgct tgccaatctg cagcaggcca 540
tgcgcaagag ccgcccctg tgttgacca tgggtggcac gctgggcccgc gagctcatga 600
tccgcccga gagaggggca ggctggaccc agcgcagag ggggtggggtg atcatacca 660
cgcgcaaggc cgtggacgcc agcagcaacg tgctgcccac cacttacagc aagttcacgg 720
agatggcggc caagggcgac accatctaca tcggccgcta cctgggtgtgc ggcgcagaca 780
gcgctcgtc gtacctggag gtcattggagc tgcagggcga cgactgtac tgcacgcca 840

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agaacgacgc ggtgctggac ggcctgctga cgggtttcca cgcggagcgc tccgtggagg 900
ggctggccaa cgtgcagaac gacctgccgc tgetgtccga ctacgacaag gagtgccctgc 960
acatcctggc gcaggacttc gagcgcgcgc cctacatctc caagctggag tccatcgctc 1020
cctccgcctg gcgcgccgc gaccgcgtgg gcgccagcct gattgtggtg tacacgcaca 1080
ccggcaagac ggcgagctg gtggccaagt accggccgcc catgcccac ctagcctgg 1140
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gccagtgctc catcagtcgc gcgctgctgc cgggtgctggc cgcgccctcg cccagcggcg 1260
accagctgct gcaggaggcg gtggccatgg cgggcccgcg caagctggtc aagcccacg 1320
accacgtggt gtgcgtgcag cgcacccacg acgactctcg cgtcaagatc atctccgtg 1380
acgacatggg cgcgggcac c aagcgcgacg acacggctcat gtcgcacagc gtgtttggca 1440
gcagcccat ggccgtgcag ggctcgtccg gctacgactc gccgcgcgtg cacaacaacc 1500
ccatcggcaa caagtccgca cccatgccgc ccgccatcat caccaccggc aatagcttca 1560
ccctgggagg catgggctgt ggctgctgtg aaatggaggc gctcgttgat ctgagccttg 1620
ccccctgacg aacggcggtg gatggaagat actgctctca agtgctgaag cggtagctta 1680
gtccccgtt tcgtgctgat cagtctttt caaacgtaa aaagcggagg agttttgcaa 1740
ttttgttggg tgtaacgate ctccgttgat tttggcctct tttccatgg gcgggctggg 1800
cgtattttaa gcggttctct cttctgccgt ta 1832

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<210> SEQ ID NO 16

<211> LENGTH: 4376

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 16, Example
16: designer HydA1-promoter-linked Pyruvate-Ferredoxin-
Oxidoreductase DNA construct (4376 bp)

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<400> SEQUENCE: 16

```

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<210> SEQ ID NO 17

<211> LENGTH: 6092

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 17, Example
17: designer HydA1-promoter-linked Pyruvate-NADP+-Oxidoreductase
DNA construct (6092 bp)

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<400> SEQUENCE: 17

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<210> SEQ ID NO 18

<211> LENGTH: 1856

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 18, Example
18: designer HydA1-promoter-linked Thiolase DNA construct
(1856 bp)

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<400> SEQUENCE: 18

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<210> SEQ ID NO 19

<211> LENGTH: 1550

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 19, Example
19: designer HydA1-promoter-linked 3-Hydroxybutyryl-CoA-
Dehydrogenase DNA construct (1550 bp)

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<400> SEQUENCE: 19

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agaaaatctg gcaccacacc gagctgtcat gcggtgttcc gttatgtgtc gtcaaacgcc 60
ttcgagcgcg gcccgaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gctggcgtgac tggctcaagc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggctcgcga taggcaagct 240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtcctccg tctccgcggc cgtggctcgc ccggcccgcg 360
ccagcgtgcg ccccatggcc gcgctgaagc ccgccgtcaa ggctgccccg gtggctgccc 420
cggctcaggc caaccagatg caaaagattt gtgtaatagg tgctggaaca atgggctcag 480
gcatcgcctc agtatttgca caaatggctt ttgaagtaat tttacgcgat attgatatga 540
agtctgtaga aaaaggattt ggcacaattg aaaaaattta caaagaaatg ttgacaaagg 600
gaaaattaca gcagatgaga aaacgaattt taagcagaat cagaggtaca acaaatctgg 660
aagacgcaaa agaagcagat tttgtagttg aagcggctat agaaaatag gatctcaaga 720
aacaaatatt caaagagcta gatgaaatg gcaaatgga aacaatcctt gcgtcaata 780
catcatcact atccataaca gaaatagcaa gtgcgacaaa aagacctgag aaagtcatag 840
gaatgcattt cttcaaccca gttccagtaa tgaaacttgt tgaagtcata aaaggattaa 900
agacatcaga gcaaacattt aatgctgca gagaattggc tttaaaagta gacaaaacac 960
ctatagaggt caaagaagca cctggatttg ttgtaaatag gattttaatc ccaatgatta 1020
atgaagcaat tggaaactct gcagtggtgt tggcaactga caagagcata gatgaagcta 1080
tgaaacttgg tgcaaatcat ccaataggac ctttggcatt gtctagtttg ataggcaatg 1140
acgtcgttct tgctataatg aatgtgcttt atgaagagta cggcgattcg aaatacagac 1200
cacatccact tctaaaaaaa gtggttaagag gcggattgct gggtagaaaa actggcaaaag 1260
gtttctttga atacaaaatt aatcttttaa ggaggagaat atcatgataa atggaggcgc 1320

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tcgttgatct gagccttgcc ccctgacgaa cggcgggtgga tggaagatac tgctctcaag 1380
tgctgaagcg gtagcttagc tccccgttc gtgctgatca gtctttttca acacgtaaaa 1440
agcggaggag ttttgcaatt ttgttggtg taacgatcct ccgttgattt tggcctcttt 1500
ctccatgggc gggctgggcg tatttgaagc ggttctctct tctgccgtta 1550

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<210> SEQ ID NO 20
<211> LENGTH: 1457
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 20, Example
20: designer HydA1-promoter-linked Crotonase DNA construct
(1457 bp)

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<400> SEQUENCE: 20

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agaaaatctg gcaccacacc gagctgtcat gcgttgttcc gttatgtgtc gtcaaacgcc 60
ttcgagcgct gcccgaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gcgggctgac tggtaaggc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggtcgcgca taggcaagct 240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtcctccg tctccgcggc cgtggctcgc ccggcccgc 360
ccagcgtgcg ccccatggcc gcgctgaagc ccgccgcaaa ggtgcccccc gtggctgccc 420
cggctcaggc caaccagatg gattttaata atgttttatt aaataaggat gatgggatag 480
ctctcatcat tataaatcgt ccaaaggctt taaatgcatt aaactatgag aactaaaaag 540
agttagatag tgtgcttgat atagttgaaa atgataaaga gataaaagtt ttaattataa 600
ctggcagcgg tgaaaaaacc ttcgtgacg gtgctgatat agctgagatg agtaaatatga 660
caccacttga agcgaagaag ttctctcttt atggacagaa agtatattagg aagatagaaa 720
tgctaagtaa gcctgttata gcagcggtaa atggttttgc acttgggtgtt ggatgacgagc 780
tttctatggc atgtgacata cgtattgcaa gtaaaaatgc aaaatttggc caacctgaag 840
taggacttgg aataatacct ggcttttcag gaactcaaag attaccacgt cttataggca 900
cttctaaagc taaagagctt attttcacag gtgacatgat aaattctgat gaagcatata 960
aaataggcct tatatctaaa gttgttgaac tatctgatct cattgaagaa gcaaaaaaac 1020
tcgcgaaaaa aatgatgtca aaaagtcaaa tagcaatttc tctagcaaaag gaagcaataa 1080
ataagggaat ggaaacagac ttagatacag gcaatactat agaagctgag aaattttcct 1140
tatgttttac aacagatgat caaaaagaag gtatgattgc gttttctgaa aagaggggcg 1200
ctaaatttgg caaataaatg gaggcgctcg ttgatctgag ccttgcccc tgacgaacgg 1260
cggtgatgg aagatactgc tctcaagtgc tgaagcggta gcttagctcc ccgtttcgtg 1320
ctgatcagtc tttttcaaca cgtaaaaagc ggaggagttt tgcaattttg ttggttgtaa 1380
cgatcctccg ttgattttgg cctctttctc catggggcgg ctgggcgtat ttgaagcgg 1440
tctctcttct gccgtta 1457

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<210> SEQ ID NO 21
<211> LENGTH: 1817
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 21, Example

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-continued

21: designer HydA1-promoter-linked Butyryl-CoA-Dehydrogenase DNA
construct (1817 bp)

<400> SEQUENCE: 21

```

agaaaatctg gcaccacacc gagctgtcat gcggtgttcc gttatgtgtc gtcaaacgcc      60
ttcagagcgt gcccggaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa     120
gcgggctgac tggcaagcgc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc     180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggtcgcgca taggcaagct     240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc     300
aaatggccgc cgtcattgcc aagtcctccg tctccgcggc cgtggctcgc ccggcccgcct     360
ccagcgtgcg ccccatggcc gcgctgaagc ccgccgtcaa ggctgccccc gtggctgccc     420
cggctcaggc caaccagatg gacttttcat taacaaagga gcaagaaatg gtaaggcgtg     480
ttgtgagaga attcgcgtgaa aaagaagttg ctcctaaagc aaaagaaata gatatcacag     540
aagagtttcc atgggataca gtaagaaaaa tggctcaaaa cgatatgatg ggtattcctt     600
atccagaaga gtatggtgga gcaggtggag attacttgag ttatatcata gctgttgaag     660
agatatcaag agcttgtgct acgactggag taattttatc tgctcactact tcattgggaa     720
gttttccaat atatcaatgg ggaacagaag aacaaaaaag aaaatatcta gtgccacttg     780
caaaagtgta aaaattgggc gcttttggcc ttacagaacc taacgcaggt acagatgcag     840
ctggacagca gacaactgca gtattagatg gtgatcacta cgtattaaac ggctcaatat     900
ttattacaaa cggagaaaaa gctgacatat atataatctt tgcaatgaca gacaaatcaa     960
aaggcacaag aggcattagt gcatttatag ttgagaaaga ttttccgggt ttagcattg     1020
gcaaaattga agaaaaaatg ggtataagag cttcatcaac tgccgaactt gtgtttgaag     1080
attgtattgt accaaaaagaa aatttacttg gtaaagaagg agaaggtttt aaaattgcca     1140
tggctacact agatggtgga agaataggaa tagcagcga acgccttggg atagctcagg     1200
ctgctttaga tgaagagata aaatatgcaa aggaaagaca acagtttgga agaccaattg     1260
gaaaatttca aggcattcaa tggatatatag ctgatatggc aacgagaata aatgcttcaa     1320
gatggcttgt atacaatgcc gcttggagaa agcaggtagg tcttccgtac acaatggaag     1380
cagctatggc aaaattatat gcttccgaaa cagcaatggt tgtaacgaca aaaacagttc     1440
agatatttgg cggctatggc tttacaaaag attatccagt ggaaagattt atgagagatg     1500
caaaaataac agaaatttat gaaggcacat cggaaagcca gaaaatggtt atttccggta     1560
acctattgaa aatgtaaagt gaggcgctcg ttgatctgag ccttgccccc tgacgaacgg     1620
cggtgatgag aagatactgc tctcaagtgc tgaagcggta gcttagctcc ccgtttcgtg     1680
ctgatcagtc tttttcaaca cgtaaaaagc ggaggagttt tgcaattttg ttggttghaa     1740
cgatcctccg ttgattttgg cctctttctc catgggcggg ctgggcgtat ttgaagcggg     1800
tctctcttct gccgtta                                     1817

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<210> SEQ ID NO 22

<211> LENGTH: 2084

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 22, Example
22: designer HydA1-promoter-linked Butyraldehyde-Dehydrogenase DNA
construct (2084 bp)

-continued

<400> SEQUENCE: 22

agaaaatctg gcaccacacc gagctgtcat gcgttgttcc gttatgtgtc gtcaaacgcc 60
ttcagagcgt gcccggaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
gcgggctgac tggcacaagg gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggtcgcgca taggcaagct 240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtcctccg tctccgcggc cgtggctcgc ccggcccgc 360
ccagcgtgag ccccatggcc gcgctgaagc ccgccgtcaa ggctgcccc gtggctgccc 420
cggtcaggc caaccagatg attaaagaca cgctagtttc tataacaaaa gatttaaaat 480
taaaaaaaa tgttgaaaat gccaatctaa agaactacaa ggatgattct tcatgtttcg 540
gagttttcga aaatgttgaa aatgctataa gcaatgccgt acacgcacaa aagatattat 600
cccttcatta taaaaagaa caaagagaaa aaatcataac tgagataaga aaggccgcat 660
tagaaaaata agagattcta gctacaatga ttcttgaaga aacacatag ggaagatag 720
aagataaaat attaaagcat gaattagtag ctaaatacac tcctgggaca gaagatttaa 780
ctactactgc ttggtcagga gataacgggc ttacagtgtg agaaatgtct ccatatggcg 840
ttataggtgc aataactcct tctacgaatc caactgaaac tgtaaatatg aatagtatag 900
gcatgatagc tgctgaaaat actgtggtat ttaacggaca tccaggcgcgt aaaaaatgtg 960
ttgtttttgc tgtcgaaatg ataaataaag ctattatttc atgtggtggc cctgagaatt 1020
tagtaacaac tataaaaaat ccaactatgg actctctaga tgcaattatt aagcacccct 1080
caataaaaact actttcggga actggagggc caggaatggt aaaaaccctc ttaaattctg 1140
gtaagaaagc tataggtgct ggtgctggaa atccaccagt tattgtagat gatactgctg 1200
atatagaaaa ggctggtaag agtatcattg aaggctgttc ttttgataat aatttacctt 1260
gtattgcaga aaaagaagta tttgtttttg agaacgttgc agatgattta atatctaaca 1320
tgctaaaaaa taatgctgta attataaatg aagatcaagt atcaaagta atagatttag 1380
tattacaaaa aaataatgaa actcaagaat actctataaa taagaaatgg gtcggaaaag 1440
atgcaaaatt attccttagat gaaatagatg ttgagtctcc ttcaagtgtt aaatgcataa 1500
tctgcgaagt aagtgcgaag catccatttg ttatgacaga actcatgatg ccaatattac 1560
caattgtaag agttaaagat atagatgaag ctattgaata tgcaaaaaata gcagaacaaa 1620
atagaaaaa tagtgoccat atttattcaa aaaatataga caacctaaat aggtttgaaa 1680
gagaaatcga tactactatc tttgtaaaga atgctaaatc ttttccggg gttggttatg 1740
aagcagaagg ctttacaact ttcactattg ctggatccac tggtaagga ataacttctg 1800
caagaaatth tacaagacaa agaagatgtg tactcgcggg ttaaatggag gcgctcgttg 1860
atctgagcct tgccccctga cgaacggcgg tggatggaag atactgctct caagtgctga 1920
agcggtagct tagctccccg tttcgtgctg atcagtcttt ttcaacacgt aaaaagcggg 1980
ggagttttgc aatthttgtg gttgtaacga tctcctgttg atthttggcct ctttctccat 2040
gggcccggctg ggcgtatttg aagcggttct ctcttctgcc gtta 2084

<210> SEQ ID NO 23

<211> LENGTH: 1733

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 23, Example
23: designer HydA1-promoter-linked Butanol-Dehydrogenase DNA
construct (1733 bp)

<400> SEQUENCE: 23

agaaaatctg gcaccacacc gagctgtcat gogttgttcc gttatgtgtc gtcacaacgcc 60
ttcgagcgct gcccgaaca atgcgtaacta gtataggagc catgaggcaa gtgaacagaa 120
gcgggctgac tggccaaggc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggtcgcgca taggcaagct 240
cgcaaatgct gtcagcttat cttacatgaa cacacaaaaca ctctcgcagg cactagcctc 300
aaatggccgc cgtcattgcc aagtcctccg tctccgcggc cgtggctcgc ccggcccgcct 360
ccagcgtgcg ccccatggcc gcgctgaagc ccgcccgtcaa ggctgcccc gtggtgccc 420
cggctcaggc caaccagatg aaagggtttg caatgctagg tattaataag ttaggatgga 480
tcgaaaaaga aaggccagtt gcggttcat atgatgctat tgtacgccc ttagcagtat 540
ctcogtgtac atcagatata catactgttt ttgagggagc tcttgagat aggaagaata 600
tgatthtagg gcatgaagct gtagtgaaag ttgtgaagt aggaagtga gtgaaggatt 660
ttaaacctgg tgacagagtt atagttcctt gtacaactcc agattggaga tctttggaag 720
ttcaagctgg ttttcaacag cactcaaacg gtatgctcgc aggatggaaa ttttcaaat 780
tcaaggatgg agtthttggt gaatttttc atgtaaatga tgcggatag aatcttgcca 840
ttctacctaa agacatgcca ttagaaaatg ctgttatgat aacagatag atgactactg 900
gatttcatgg agcagaactt gcagatattc aaatgggttc aagtgttggt gtaattggca 960
ttggagctgt tggcttaatg ggaatagcag gtgctaaatt acgtggagca ggtagaataa 1020
ttggagtggt gagcaggccg atttgtgttg aggctgcaaa attttatgga gcaacagata 1080
ttctaaatta taaaatggt catatagttg atcaagttat gaaattaacg aatggaaaag 1140
gogttgaccg cgtaattatg gcaggcggtg gttctgaaac attatcccaa gcagtatcta 1200
tggttaaacc aggaggaata atttctaata taaattatca tggaaagtga gatgctttac 1260
taataccacg tgtagaatgg ggatgtgaa tggctcacia gactataaaa ggaggtcttt 1320
gtcctggggg acgtttgaga gcagaaatgt taagagatat ggtagtatat aatcgtgttg 1380
atctaagtaa attagttaca catgtatata atggatttga tcacatagaa gaagcactgt 1440
tattaatgaa agacaagcca aaagacttaa ttaaagcagt agttatatta taaatggagg 1500
cgctcgttga tctgagcctt gcccctgac gaacggcggg ggatggaaga tactgctctc 1560
aagtgtgaa gcggtagcct agctcccctt ttcgtgctga tcagtctttt tcaacacgta 1620
aaaagcggag gagthttgca atttgtgttg ttgtaacgat cctccgttga tttggcctc 1680
tttctccatg gcggggctgg gcgtatttga agcgggtctc tctctgccc tta 1733

<210> SEQ ID NO 24

<211> LENGTH: 1556

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 24, Example
24: designer Fructose-Diphosphate-Aldolase DNA construct (1556 bp)

<400> SEQUENCE: 24

agaaaatctg gcaccacacc atggtagggt gogagtgacc ccgcccact tggaaagggt 60

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caaacgacct cgccgtacga acttttgcg gggggcgctc ccggatgta ggggctgagt 120
gaccccgcg cacttggaag ggttcaaacg accccgcegt acgaactttt gtcggggggc 180
gtcccggat ggcctgatg atgaagtcgt cggccagcct gaaggctgtg tcgctggccg 240
ctctcgccg gccgtcgtt ggcgcgccg gcaagtacga tgaggagctg attaagaccg 300
ctggcaccgt tgctccaag ggcgcggta tctggccat ggacgagta aacgccacct 360
gcgcaaacg cctggactcc atcggcgtg agaacaccga ggagaaccgc cgcgcctacc 420
gagagctgct ggtgaccgc cccggcctg gccagtaac ctcggcgtc atcctgttcg 480
aggagaccct gtatcagtc accgcctcc gcaagaagt cgtcgtatg atgaaggagc 540
agaacatcgt gcccggcctc aaggtcgaca agggcctggt gccctgtcca acaccaacga 600
tgagctggtg catgggctg gacggctgga caagcgtgc tgagtactac aaggccggcg 660
ctcgtctcg caagtggcgc tcggtcgtc cgatccccc cggcccctc atcatgctgc 720
cgcgactgg ctacggcctg gccgcctac ccgccatcgc ccagaaccgc ggtctggtgc 780
ccattgtgga gcccaggtc ctgctggacg gtgagcacga catcgaccgc tgcctggagg 840
tgaggaggc catctgggc gagacctca agtacctgc cgacaacaag gtcctggtgc 900
agggtatcct gctgaagccc gccatggtc ccccggcgc tgactgcaag aacaaggccg 960
gccccccaa ggttgccgag tacacctga agatgctggc cgcgcgtgc ccccggctc 1020
ccggcatcat gttcctgct ggcggccagt ccgagctgga gtcgacctg aacctgaacg 1080
ccatgaacca gagccccaac ccgtggcagc tgcctgttc gtacgccgc gctctgacga 1140
acaccgttct gaagacctg caggcaagc cgagaacgt ccaggcgc ccaggctcgtg 1200
ctcaagcgc caagccaac tcggacgctc agcaggcga gtacgacgc accaccagg 1260
gcaaggaggc tgcccaggc atgtacgaga agggaaaagg ctacgtctac taataaatgg 1320
aggcgtcgt tgatctgagc cttgccccct gacgaaccgc ggtggatgga agatactgct 1380
ctcaagtct gaagcgtg cttagctccc cgttctgctc tgatcagctc tttcaacac 1440
gtaaaaagcg gaggatgtt gcaatttgt tgggtgtaac gatcctcgt tgattttggc 1500
ctctttctcc atggcgggc tggcgctatt tgaagcgtt ctctctctc cgtta 1556

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<210> SEQ ID NO 25

<211> LENGTH: 1379

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 25, Example 25: designer Triose-Phosphate-Isomerase DNA construct (1379 bp)

<400> SEQUENCE: 25

```

agaaaatctg gcaccacacc atggtagggg gcgagtgacc ccgcgcgact tggagggtt 60
caaacgacct cgccgtacga acttttgcg gggggcgctc ccggatgta ggggctgagt 120
gaccccgcg cacttggaag ggttcaaacg accccgcegt acgaactttt gtcggggggc 180
gtcccggat ggcagctacc tctctcaact cccctcctc tttctccgg ctcgcccga 240
tttctccaa getcgacgt gccgcgtct cctcccacca atccttctc caccgcgtc 300
attcctctac ccgtctcgt tcttctctt cttcttctc tcgctcccc agagggttg 360
ttgccatggc tggatccgga aagtttttcg ttggaggaaa ctggaagtgt aacgggacta 420
aggactccat cgccaagctt atctcogac tcaacagtgc aaccttgaa gcagatgtag 480

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atgttggtgt gtcacctcca tttgtctaca tgcaccaggt caaatcctcg ttgacagacc 540
gtattgacat atcaggtcag aactcttggg ttgggaaagg tggagccttc actggtgaaa 600
tcagcgtgga acagctcaaa gaccttggct gcaagtgggt cattcttggg cattccgaac 660
ggagacatgt catcggagaa aaagatgagt ttatcgggaa gaaagctgca tatgcattga 720
gtgagggtct tggagtgata gcttgtattg gggaaaagct agaagagagg gaagcaggca 780
agacgtttga tgtttgcttc gcgcaactga aggcgtttgc tgatgctgtg cctagctggg 840
acaatatagt tgttgcatac gagcctgtat gggcaattgg aactggtaaa gttgcatctc 900
ctcagcaagc acaagaagtc catgtagctg tccgcggttg gctaaagaag aatgtctctg 960
aggaagttgc ttccaaaacg agaatcatat atggaggttc tgtcaatgga ggcaacagtg 1020
cagagcttgc caaagaagaa gacattgatg gatttcttgt tgggtgtgcc tccttgaagg 1080
gtcctgagtt tgcaaccatt gtgaactcag tcacgtcga gaaagttgct gcttgataaa 1140
tggaggcget cgttgatctg agccttggcc cctgacgaac ggcggtggat ggaagatact 1200
gctctcaagt getgaagcgg tagcttagct ccccgtttcg tgctgatcag tctttttcaa 1260
cacgtaaaaa gcgaggaggt tttgcaattt tgttggttgt aacgatctc cgttgatttt 1320
ggcctcttcc tccatgggag ggctgggagc atttgaagcg gttctctctt ctgccgtta 1379

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<210> SEQ ID NO 26

<211> LENGTH: 2156

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 26, Example
26: designer Phosphofructose-Kinase DNA construct (2156 bp)

<400> SEQUENCE: 26

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtcgagt 120
gaccccgcgc gacttggaa ggttcaaacg accccgccgt acgaactttt gtcggggggc 180
gtcccccgat ggccgcccgc attgccaaat cctccgtctc cgccggccgtg gctcggcccg 240
cccgtcccag cgtgcgcccc atggccgccc tgaagcccgc cgtcaaggct gcccccgtgg 300
ctgccccggc tcaggccaac cagatggaag cttcgatttc gtttctgggg tcaacaaaac 360
ccaatatctc cttgtttaac ccttcttcaa acgctcctcc tcgtagagat ttcctcttc 420
ctgctttgaa attgaagaaa gtttcagtgc tgcctcgaat cttgcaccag aaacgactca 480
tcagagctca gtgctctgat ggattcaaac cagaggaaga cgatgggttt gtccatagaag 540
acgttctctc cttgacaaa tttctccctg atttaccgtc atatccaaat ccattgaaag 600
aaagccaagc atatgccatt gtaagcgaa cttttgtcag ttccgaagat gtggttgccg 660
aaaaatattg agtccagaag ggaagtaagc gaggagtaca ctttaggcga gcagggcctc 720
gagaaagagt gtacttcaga tcagatgaag taaaagcttg catagtgact tgtgggggct 780
tgtgccctgg aatcaatact gttatcggg aaattgtatg tggattgaac aatattgtatg 840
gtgttaataa cattctcgcc attcagggag gatatagagg cttttactcc aaaaacacta 900
tgaacctgac acctaaagta gttaacgata ttcataaacg cggtggcact tttcttcaaa 960
cctcaagagg aggacatgat acagcgaaga ttgttgataa tattcaagat agaggaataa 1020
atcaggtata tattattgga ggtggtggga cgcaaaaggg tgcagagaag atatacgagg 1080

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aagttgagag gcgtggtcct caagtggcgg tttctggcat tcctaagaca attgataatg 1140
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ctattaatgc tgcacatgta gaggtcgaga gcgtggaaaa tggagttggt atcgtaaacc 1260
tcatgggcag atacagtgtt tttattgcca tgattgcaac tttagcgaat cgtgatgtgg 1320
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ttattgaaga acgactcaaa gagaataggc acatggttat tgtgatagct gaaggagctg 1440
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gactcttgct tgatgttggt ctatggttga ctcaacagat aaaggatcac tttacaatg 1560
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caatggctgg gtactcaggt ttcactgtag gaccagttaa cagtagacat gcttacatcc 1740
caattctctg gacggaagtg acaaatatcg tgaagttaac tgataggatg tgggctagac 1800
tccttgcatc gacaaatcaa ccgagtttct tgactggtga aggagcattg cagaatgtga 1860
tcgacatgga aactcaagaa aagatcgata acatgaagat ctcttctatc taataaatgg 1920
aggcctcctg tgatctgagc cttgccccct gacgaacggc ggtggatgga agatactgct 1980
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gtaaaaagcg gaggagtttt gcaattttgt tgggtgtaac gatcctcctg tgattttggc 2100
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<210> SEQ ID NO 27

<211> LENGTH: 860

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 27, Example
27: designer N1a1-promoter-linked Starch-Synthase-iRNA DNA
construct (860 bp)

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<400> SEQUENCE: 27

```

agaaaatctg gcaccacacc tatatggtag ggtgcgagtg accccgcgcg acttgagact 60
cgatggcccc gggttgtttg gggcgteccg ctctcgcgct attctgagct ggagaccgag 120
gcgcatgaaa atgcattcgc ttccatagga cgctgcattg tggcttgaag gttcaaggga 180
agggttcaaa cgacccccgc gtacgaactt ttgtcggggg gcgctcccgg ccccgggctc 240
ttgtgcgcgc attagggcct cgggtcgcaa gcaagacgat acatgccagc cggctcacca 300
ccgccaccag cggcttgccg gggctccact ccaggcccag acccttctgc agaaactcct 360
tgcacagcgc cttcccggcg gggcggctcg cgtcgaagtt ggtggcagc agcgcgtcag 420
tggccggggt ccaactctca cagtcaatgc cgttcaggat gccgtggaac ttggagcgca 480
gctcggggcg cgcaagggtg gatctcgccg tcccagcggg agcccttggg cacctcgatg 540
tcgcattcgt gcttgaggcc ctcaatctgg tccttgggca ggcactcgta gaacggcagc 600
atgacctgca cgaagtgtaa atggagcgcg tcgttgatct gagccttggc cctgacgaa 660
cggcgggtga tggaaagatac tgctctcaag tgctgaagcg gtacgttagc tccccgttcc 720
gtgctgatca gtctttttca acacgtaaaa agcggaggag ttttgcaatt ttgttggtg 780
taacgatact ccgttgatgt tggcctcttt ctccatgggc gggctggggc tatttgaagc 840

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 ggttctctct tctgcccgtta 860

<210> SEQ ID NO 28
 <211> LENGTH: 1328
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 28, Example
 28: designer HydA1-promoter-linked Starch-Synthase-iRNA DNA
 construct (1328 bp)

<400> SEQUENCE: 28

agaaaatctg gcaccacacc gagctgtcat gcggtgttcc gttatgtgtc gtcaaaacgcc 60
 ttcgagcgct gcccgaaca atgcgtacta gtataggagc catgaggcaa gtgaacagaa 120
 gcgggctgac tggtaaggc gcacgatagg gctgacgagc gtgctgacgg ggtgtaccgc 180
 cgagtgtccg ctgcattccc gccggattgg gaaatcgcga tggctgcgca taggcaagct 240
 cgcaaatgct gtcagcttat cttacatgaa cacacaaaaca ctctcgcagg cactagcctc 300
 aatgaagag cttcatgcgg agggatgcgc tcggcgcggg gctccgcggg gcagccagca 360
 caaagcccgt ctcaagggtc gccagcgtga ggctgcgcc tacccgctac cgcactgcct 420
 gccaaagtgc gaaggtggat gaaatggtgt cgggtgatga ggagcttact cgtctccgca 480
 aggagaacga gctcctgcgc gcccaactgg cgctgtacca gcagaaccag cagccgtccg 540
 tgggtgccgc tgccgttgcc ccgctgctg ccgccacgaa ggtgctggag aagccggcgc 600
 cgtaagtaac ctaacggtga gcagcatgca atattttagc gtcgatactc gaaactata 660
 ggagcgcctc agccgaccga tgttcgcggt gctgtcgcag gcccaaccgt gccaccgccg 720
 tgggtgtgca ggcgcagaag gggccaggc ccgctgctg gctgctctgg ccataagtaa 780
 cctaaccggc ccggtctctc cagcaacctc gtggcggcag caggcggggc aacggcagcg 840
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 ggctttgtgc tggctgcacc gcggagcccc gcgccgagcg catccctccg catgaaactc 1080
 ttcattaaat ggaggcgctc gttgatctga gccttgcccc ctgacgaacg gcggtggatg 1140
 gaagatactg ctctcaagtg ctgaagcggg agcttagctc cccgtttcgt gctgatcagt 1200
 ctttttcaac acgtaaaaag cggaggagtt ttgcaatttt gttggttga acgatcctcc 1260
 gttgattttg gcctctttct ccatgggccc gctggggcgt tttgaagcgg ttctctcttc 1320
 tgccgtta 1328

<210> SEQ ID NO 29
 <211> LENGTH: 1889
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 29, Example
 29: designer Amylase DNA construct (1889 bp)

<400> SEQUENCE: 29

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
 caaacgacct cgcctacga acttttctc gggggcgctc ccggatgta ggtgacgagt 120
 gaccccgccg gacttgaag ggttcaaacg accccgcccg acgaaacttt gtcggggggc 180

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gctcccggct cgagcatatg gccgcgctca ttgccaagtc ctccgtctcc gcggccgtgg 240
ctegcccggc ccgctccagc gtgcgcccga tggccgcgct gaagcccgcc gtcaggctg 300
cccccgctggc tgccccggct caggccaacc agatggcgaa caaacacatg tccctttctc 360
tcttcatcgt cctccttggc ctctcgtgca gcttggcctc cgggcaagtc ctgtttcagg 420
gttttaactg ggagtcgtgg aagcacaatg gcgggtggta caacttctg atgggcaagg 480
tggacgacat cgcgcgctg ggcgtcacgc acgtgtgget ccccccgcg tcgcagtccg 540
tcgccgagca aggttacatg ccgggcccgc tctacgacct ggacgcctcc aagtacggca 600
acaaggcgca gctcaagtcc ctcatcggcg cgctccaagg caaggcgctc aaggccatcg 660
ccgacatcgt catcaaccac cgcacggcgg agcgcaagga cggccggggc atctactgca 720
tcttcgaggg cggcaccctg gacgcgcgcc tcgactgggg cccccacatg atctgcccgc 780
acgaccggcc ctacgcggac ggcaccggca acccggacac cggcgccgac ttcggggccg 840
cgccggacat cgaccacctc aaccgcgcgc tccagaagga gctcgtcgag tggtcaact 900
ggctcaggac cgacgtcggc ttcgacggct ggcgcttcga cttcgccaag ggtaactccg 960
cggacgtggc caagatctac gtcgaccgct ccgagcccag cttcgccgct gccgagatat 1020
ggacgtcgtg ggcgtacggc ggggaocggc agccgaacct caaccaggac ccgaccggc 1080
aggagctggt gaactgggtg aacaagggtg gcggtccgg ccccgccacc acgttcgact 1140
tcaccaccaa gggcatcctc aacgtggcgc tggagggcga gctgtggcgc ctgcccggca 1200
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tacacagtga gagcaagctg cagatcatgg aggcgacgc cgacctttac cttgcccaga 1500
tcgacggcaa ggtcatcgtc aagctcgggc caagatacga tgtcggacac ctcatctctg 1560
aaggctcaa ggtggtcgcg catggcaatg actatgccgt atgggagaaa gtataaggct 1620
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ccccgttctg tctgatcag tctttttcaa cacgtaaaaa cggaggagt tttcaattt 1800
tgttggtgtt aacgatcctc cgttgatttt ggcctcttcc tccatgggcg ggtgggctg 1860
atttgaagcg gttctctctt ctgccgtta 1889

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<210> SEQ ID NO 30

<211> LENGTH: 3089

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 30, Example 30: designer Starch-Phosphorylase DNA construct (3089 bp)

<400> SEQUENCE: 30

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaagggtt 60
caaacgaccc cgcctacga acttttctg gggggcgctc ccggatggta ggtgctgagt 120
gaccccgcgc gacttgaag ggttcaaacg accccgcgct acgaactttt gtcggggggc 180
gctcccggat ggcgcccgtc attgccaagt cctcgtctc cgggcccgtg gctcggccg 240

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cccgtccag cgtgcgcccc atggcgcgc tgaagcccgc cgtcaaggct gccccgtgg	300
ctgccccggc tcaggccaac cagatggcgg atgcgaaagc aaacggaaag aatgaggcgg	360
ccaaactggc gaaaattccg gcggctgcga atccattggc taatgaacca tcggcgattg	420
catcaaatat aagttaccac gtgcagtaca gtcctcattt ctgcgcgact aagttcgagc	480
cggagcaagc tttctttgcc acggcggagg ttgtccgcga tcgtcttatt caacaatgga	540
atgagacata ccaccatttt aataaagttg atccgaagca aacatactac ctatcaatgg	600
aatttcttca aggaaggact ttgactaatg caattggcag tttggacatt cagaatgcat	660
atgctgatgc tttaaataat ttggggcatg tccttgagga gatagctgaa caggaaaaag	720
atgctgcaat aggaatggg gggctgggca ggctagcttc atgcttctta gactccatgg	780
caacattgaa tttgcctgca tggggttatg gtttgagata ccggtatggg ctgttcaagc	840
agaagatcac caagcagggt caagaagaag ttgctgaaga ttggcttgag aaatttagtc	900
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ttaatccaaa tggaaacgaga aaatgggttg ggggtgaagt tgtccaagcc gtagcttatg	1020
atataccaat tccagggtac aaaaccaaga acaactatcag tcttcgtctc tgggacgcta	1080
aagctagcgc tgaggatttc aatttatctc agttaaataa tggacaatac gaatctgctg	1140
cacagcttca ttctcgagct caacagattt gtgctgtgct ctacccccgg gatttctactg	1200
aagaagggaa gcttttaagg ctgaaacaac aattctttct ctgcagtgct tcaactcagg	1260
atagatttct tagattcaag gagaggaaaa gtggaaggca gtggtctgaa tttccagca	1320
aggtagctgt acaactgaat gatactcatc caacacttgc aattccagag ttgatgcgat	1380
tgctaataag tgaggaaagg cttggatggg atgaagcatg ggatataaca acaaggactg	1440
ttgcttatac caatcacaca gtacttctcg aagcacttga gaagtggta caagcagtaa	1500
tgtagaagct tcttctctgc catatggaaa taattgaaga gattgacaag agattcattg	1560
caatggtccg ctccacaagg agtgacctg agagtaagat tccagcatg tgcactctgg	1620
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cggtaaatgg tgttctctag ttgcacagt atactttaa ggccgacttg ttcgctgact	1740
atgtttctct atggccaaac aaactccaaa ataaaactaa tggcattact cctcgtcgat	1800
ggctccgggt ttgcaatcct gagctcagca aaattatcac aaaatggta aaaaccgatc	1860
agtgggttac gaaccttgac ctgctttag gtctctctca gtttctgac aacacagaac	1920
tccaagctga atgggaatct gctaagatgg ccagtaagaa acatttggca gactacatat	1980
ggcagtaac cgggtgaacg attgatccta atagcttatt tgacatacaa gtaaacgca	2040
ttcatgaata caagagacaa ctgctaaata ttttgggcgc aatctacaga tacaagaagt	2100
tgaaggagat gagccctcag gagcgggaaga aaactactcc acgcaccatt atgtttggag	2160
ggaaagcatt tgcaacatat acaaacgcaa aaagaatagt aaagtgggtt aatgatgttg	2220
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attacaatgt ctctgttgcg gagttgctta ttccaggaag tgagctatct cagcatatta	2340
gcacagcagg catggaggca agtggcacia gcaacatgaa attttctcta aatgggtgcc	2400
tcattatagg aacattggat ggagctaagt tggaaatcag gcaggagata ggagaggaga	2460
atctctttct ctttgggtgca ggagcagacc aagtccctaa gctgcgggaag gaaagagaag	2520
atggattggt caaacagat cctcgggttg aagaggccaa gcaatttata agaagtggag	2580

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catttggaag ctatgactac aaccgccttc ttgattccct ggaggggaac actgggttatg 2640
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gagttgacca agcttacaag gaccggaaga agtggctgaa gatgtctata ttaagtacag 2760
ctggcagtgg gaaattcagc agtgatcgca caattgcaca gtatgctaag gaaatctgga 2820
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cctgacgaac ggccggtgat ggaagatact gctctcaagt gctgaagcgg tagcttagct 2940
ccccgtttcg tgctgatcag tctttttcaa cacgtaaaaa gcggaggagt tttgcaattt 3000
tgttggttgt aacgatcctc cgttgatttt ggccctcttc tccatgggcg ggctgggctg 3060
atttgaagcg gttctctctt ctgccgtta 3089

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<210> SEQ ID NO 31

<211> LENGTH: 1949

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 31, Example 31: designer Hexose-Kinase DNA construct (1949 bp)

<400> SEQUENCE: 31

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtcgagt 120
gacccccgcg gacttggaa ggttcaaacg accccgcctg acgaaacttt gtcggggggc 180
gtccccggat ggccgcctgc attgccaagt cctccgtctc cgccggccgtg gctcgcccgg 240
ccccgtccag cgtgcgcccc atggccgcgc tgaagccgcg cgtcaaggct gcccccgtag 300
ctgccccgcg tcaggccaac cagatggcta taacaccocg ccgaaaacct tcccggaagg 360
gatcaatggc tgatatgccc aaggatgtgc ttgaccagct caagacgctg gaagagctct 420
tcacagttga ccaggagaag ctgaagcaga tcggtgagca tttcatcaag gagttacaga 480
agggcctcag tgtcgaagge ggaaacattc ccatgaacgt gacttggggt ctgggatttc 540
ccactggcca tgagaaaagt acatttctgg ctctggacat ggggggcacc aacctgcgcg 600
tctgcgaaat tgagctctcc gaagagaagg gcgagtttga tgtcacacag tccaagtatc 660
gaatccccga agagctcaag agcggtgaat catcagaact atgggaatat attgccgact 720
gtgtacagca gttcatagaa tactaccatg acggttgcac ggctttgcca gacctgccc 780
tgggctttac cttttcgtac cctgctactc aagaatatgt tgaccacggt gtctacaga 840
gatggaccaa gggttttgat attgacggcg tcgagggcaa agacgtcgtc ccaatgttag 900
aagaagcttt ggctaagaag gttaaaaatt cagctcttcc cccatttttc tttggctata 960
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gcacaggcgt caacgcgcc tacatggaat atgcgggctc tatccctaaa atagcccact 1140
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aactcattgt cctccccga acgcagtatg acgacgtatc ccaactacgt aaaccatact 1260
ccctggactc ctccctccta gccttcatcg aagaagatcc cttegagaac ctgtcagaaa 1320
cgcgagatct cttcgaacgc accctgggga tctacgcatt gccctcggag ctagaattct 1380
gcagacgcct ggccgaattg atcggcacac gtgccgcacg cctctccgct tgcggtgttg 1440

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cggccatctg caagaagaaa aatatcacc attgccatgt cggagcggac gggtcggtgt 1500
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ggccagatag tgaaccggat cgggttgta tgagcggagc ggaggatggg tctggcggtg 1620
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cctgacgaac ggcggtggat ggaagatact gctctcaagt gctgaagcgg tagcttagct 1800
ccccgtttcg tgctgatcag tctttttcaa cacgtaaaaa gcggaggagt tttgcaattt 1860
tgttggtgtg aacgatcctc cgttgatttt ggcctcttcc tccatgggag ggcggggcgt 1920
atttgaagcg gttctctctt ctgccgtta 1949

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<210> SEQ ID NO 32

<211> LENGTH: 2249

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 32, Example 32: designer Phosphoglucomutase DNA construct (2249 bp)

<400> SEQUENCE: 32

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtcgagt 120
gacccccgcg gacttggaa ggttcaaacg acccccgctg acgaactttt gtcggggggc 180
gtccccggat ggccgcctc attgccaaat cctccgtctc cgccggccgtg gctcgccccg 240
ccccgtccag cgtgcgcccc atggccgctc tgaagcccg cgtcaaggct gcccccggtg 300
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agcctcacta cactgaaaac ttcattcagg ctattctcga tgccattccg gaagggtccc 480
aagggtccac tcttgttgta ggaggtgatg gccgtttcta caacgacaag gtcaccaact 540
tgatcgccaa aatcgccctg gccaacggag tttccaagtt gattttgggt caagacggga 600
ttctttccac tccagcaact tcgcatgtaa tcaggatcag ggggtgcaact ggaggaatta 660
ttctcactgc ttcacacaac cccggaggcc ccaaaaaaga tttgggtatt aagtacaact 720
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aattgacttc gtacaagctc attgatttac ccgacattga tttgtccaaa acccagaccg 840
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cctctggagc catcgatttg gtagcaaaag ctaaaggatt gaatgtttac gaagtgccaa 1380
ccggttgaaa gttctctcgc aaccttttcg acgctgacaa gttgagtacc tgtggtgaag 1440

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agtcgtttgg aacaggtcc aaccacatca gagaaaagga cggcctttgg gctgtagttg 1500
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ctgtagtgca gaactcgttt tggaagaaat acggaagaac tttcttctact agatatgact 1620
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aaagcggtct gagattcata gtaagattgt ctggtagctg atcatccggt gctacagtca 1860
gattatatct cgaaaagcac tctgctgacg aatccaccta tggcttaggc gtagaccagt 1920
acttagttga tgacatcaag tttgtcttgg acttgttgaa gttcaagcag ttcttgggaa 1980
aggatgaacc agatgttctg acctagtaaa tggaggcgtc cgttgatctg agccttgccc 2040
cctgacgaac ggcggtggat ggaagatact gctctcaagt gctgaagcgg tagcttagct 2100
ccccgtttcg tgctgatcag tctttttcaa cacgtaaaaa gcggaggagt tttgcaattt 2160
tgttggttgt aacgatcctc cgttgatttt ggctctttc tccatgggcg ggctgggctg 2220
attgaagcg gttctctctt ctgccgta 2249

```

<210> SEQ ID NO 33

<211> LENGTH: 2231

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 33, Example
33: designer Glucosephosphate-Isomerase DNA construct (2231 bp)

<400> SEQUENCE: 33

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgctcgact tgaagggtt 60
caaacgaccc cgcctgacga acttttctcg gggggcgtc ccggatggtg ggtgctgagt 120
gaccccgctc gacttgaag ggttcaaacg accccgctc acgaaacttt gtcggggggc 180
gtccccgat ggcgcctc attgccaagt cctccgtctc cgcggcctg gctcggcccg 240
cccgctccag cgtgcgcccc atggcgcgc tgaagccgc cgtcaaggct gccccctgg 300
ctgccccgct tcaggccaac cagatgtcca ataactcatt cactaacttc aaactggcca 360
ctgaattgcc agcctggtct aagttgcaaa aaatttatga atctcaagg aagactttgt 420
ctgtcaagca agaattccaa aaagatgcca agcgttttga aaaattgaac aagactttca 480
ccaactatga tggttccaaa atcttcttct actactcaaa gaacttggtc aacgatgaaa 540
tcattgctgc atgattgaa ctggccaagg aggctaactg caccggtttg agagatgcta 600
tgttcaaagg tgaacacatc aactccactg aagatcgtgc tgtctaccac gtcgattga 660
gaaacagagc taacaagcca atgtaacttg atggtgtcaa cgttgctcca gaagtcgact 720
ctgtcttgaa gcacatgaag gagttctctg aacaagttcg ttctggtgaa tgaagggtt 780
ataccggtaa gaagatcacc gatgttgta acatcggtat tgggtgttcc gatttgggtc 840
cagtcattgt cactgaggct ttgaagcact acgctggtgt cttggatgct cacttcgttt 900
ccaacattga cgttactcac attgtgaaa ccttgaagg tgttgaccca gaaactactt 960
tgtttttgat tgcttccaa actttcacta ccgctgaaac taccactaac gctaacactg 1020
ccaagaactg gttcttctcg aagacaggta atgatccatc tcacattgct aagcatttct 1080
ctgctttgtc cactaacgaa accgaagttg ccaagttcgg tattgacacc aaaacatgt 1140

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ttggtttcga aagttgggtc ggtggtcgtt actctgtctg gtcggctatt ggtttgtctg 1200
ttgccttgta cattggctat gacaactttg aggctttctt gaagggtgct gaagccgctc 1260
acaaccactt caccacaacc ccattggaag acaacattcc attgttgggt ggtttgttgt 1320
ctgtctggta caacaacttc tttggtgctc aaaccattt ggttgctcca ttcgaccaat 1380
acttgacag attcccagcc tacttgcaac aattgtcaat ggaatctaac ggtaagtctg 1440
ttaccagagg taacgtggtt actgactact ctactggttc tatcttggtt ggtgaaccag 1500
ctaccaacgc tcaactctct ttcttccaat tggttcacca aggtaccaag ttgattccat 1560
ctgatttcat cttagctgct caatctcata acccaattga gaacaaatta catcaaaaga 1620
tgttggtctc aaacttcttt gctcaagctg aagctttaat ggttggttaag gatgaagaac 1680
aagttaaggc tgaagggtgc actggtggtt tgggtccaca caaggctctc tcaggtaaca 1740
gaccaactac ctctatcttg gctcaaaaga ttactccagc tactttgggt gctttgattg 1800
cctactacga acatgttact ttcaactgaag gtgccatttg gaatatcaac tctttcgacc 1860
aatgggggtg tgaattgggt aaagtcttgg ctaaagtcac cggcaaggaa ttggacaact 1920
cctccaccat ttctaccac gatgcttcta ccaacggttt aatcaatcaa ttcaaggaat 1980
ggatgtgata aatggaggcg ctgcttgatc tgagccttgc ccctgacga acggcgggtg 2040
atggaagata ctgctctcaa gtgctgaagc ggtagcttag ctccccgttt cgtgctgatc 2100
agtctttttc aacacgtaaa aagcggagga gttttgcaat tttgttggtt gtaacgatcc 2160
tccgttgatt ttggcctctt tctccatggg cgggctgggc gtatttgaag cggttctctc 2220
ttctgccgtt a 2231

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<210> SEQ ID NO 34

<211> LENGTH: 1709

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 34, Example
34: designer oxyphotobacterial Butanol Dehydrogenase DNA construct
(1709 bp)

```

<400> SEQUENCE: 34

```

agaaaatctg gcaccacacc atccaaaactc gccacccgca aaccaatggc atggccgagc 60
gctgcaacgg tcgcattgcc aagattctgc gtgctgagcg ctttgtctcc gctgctgatc 120
tgcaagagac gctcacgcga tacctctggg cgtgcaatca ccgcattccc caacgcgctt 180
tgggccacat gacccccatc gagagactcc gaacgtggca aatggagggg ccagagttgt 240
tcagttcaca ggtagataat gtcgctgggtc ttgatagtta gcaataaata cagtttcaga 300
atatctgtaa tacaaaaact gtatcgagac aagaaaaaag tagcaaaatt tacaatggt 360
catgattcat ctggctaaaat tggatgttca actgacccat tgaagacaag ggcaacaacc 420
atggagaatt ttagatttaa tgcatataca gagatgcttt ttggaaaagg acaaatagag 480
aagcttccag aggttttaaa aagatatggt aaaaatatat tacttgcata tggtggtgga 540
agtataaaaa agaattggact ctatgatact atccaaaagc tattgaaaga ttttaattat 600
gttgaattaa gtggtattga accaaatcca agaattgaaa ctgtaagacg tggagttgaa 660
ctttgcagaa aaaataaagt agatgttatt ttagctgttg gtggaggagag tacaatagac 720
tgctcaaagg ttataggggc aggttattat tatgctggag atgcatggga ccttgtaaaa 780

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aatccagcta aatataggtga ggttttacca atagtgcag ttttaacaat ggcagctact 840
ggttctgaaa tgaatagaaa tgctgttatt tcaaagatgg atacaaatga aaagcttgga 900
acaggatcac ctaagatgat ccctcaaaact tctatttttag atccagaata tttgtataca 960
ttgccagcaa ttcaaacagc tgcaggttgt gctgatatta tgtcacacat atttgaacaa 1020
tattttaata aaactacaga tgcttttgta caagataaat ttgcggaagg tttggtgcaa 1080
acttgataaa aatattgccc tgttgcttta aaggaaccaa agaattatga agctagagca 1140
aatataatgt gggctagtgc aatggctctt aacggacttt taggaagtgg gaaagctgga 1200
gcttggaact gtcacccaat agaacatgaa ttaagtgcac tttatgatat aactcatgga 1260
gtaggtcttg caattttaac tccaagttgg atgagatata tcttaagtga tgtaacagtt 1320
gataagtttg ttaacgtatg gcatttagaa caaaaagaag ataaatttgc tcttgcaaat 1380
gaagcaatag atgcaacaga aaaattcttt aaagcttggtg gtattccaat gactttaact 1440
gaacttgaaa tagataaagc aaactttgaa aagatggcaa aagctgcagt agaacatggt 1500
gctttagaat atgcatatgt ttcattaat gccgaggatg tatataaaat tttagaaatg 1560
tccctttaat aaggctgaga tcttcttcag tgcattgtag ttgaatgaag ggttaggggg 1620
gaaatgcccc cctatttttt gtctagccat cctgccacgt ttgacagggt agcaatttcg 1680
acacgatagg gttctctctt ctgccgta 1709

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<210> SEQ ID NO 35

<211> LENGTH: 1967

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 35, Example
35: designer oxyphotobacterial Butyraldehyde Dehydrogenase DNA
construct (1967 bp)

```

<400> SEQUENCE: 35

```

agaaaatctg gcaccacacc atccaaactc gccaccgca aaccaatggc atggccgagc 60
gtgcaacgg tcgcatgtgc aagattctgc gtgctgagcg ctttctctcc gctgctgatc 120
tgcaagagac gctcacgcca tacctctggg cgtgcaatca ccgcatccc caacgcgctt 180
tgggccacat gacccccatc gagagactcc gaacgtggca aatggagggg ccagagttgt 240
tcagttcaca ggtagataat gtcgcgggtc ttgatagta gcaataaata cagtttcaga 300
atatctgtaa tacaaaaact gtatcgagac aagaaaaaag tagcaaaatt tacaaatggt 360
catgattcat ctggctaaat tggatgttca actgacccat tgaagacaag ggcaacaacc 420
atgattaaag acacgctagt ttctataaca aaagatttaa aattaaaac aatggtgaa 480
aatgccaatc taaagaacta caaggatgat tcttcatggt tcggagtgtt cgaaaatggt 540
gaaaatgcta taagcaatgc cgtacacgca caaaagatat tatcccttca ttatacaaaa 600
gaacaaagag aaaaaatcat aactgagata agaaaggccg cattagaaaa taagagatt 660
ctagctacaa tgattcttga agaaacacat atgggaagat atgaagataa aatattaaag 720
catgaattag tagctaaata cactcctggg acagaagatt taactactac tgcttggtca 780
ggagataaac ggcttacagt tgtagaaatg tctccatag gcgttatagg tgcaataact 840
ccttctacga atccaactga aactgtaata tgtaaatgta taggcatgat agctgctgga 900
aatactgtgg tatttaacgg acatccagcc gctaaaaaat gtgttgcttt tgctgtcgaa 960
atgataaata aagctattat ttcattgtgt ggtcctgaga atttagtaac aactataaaa 1020

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aatccaacta tggactctct agatgcaatt attaagcacc cttcaataaa actactttgc 1080
ggaactggag ggccaggaat ggtaaaaacc ctcttaaatt ctggtaagaa agctataggt 1140
gctggtgctg gaaatccacc agttattgta gatgatactg ctgatataga aaaggctggt 1200
aagagtatca ttgaaggctg ttcttttgat aataatttac cttgtattgc agaaaaagaa 1260
gtatttgttt ttgagaacgt tgcagatgat ttaatatcta acatgctaaa aaataatgct 1320
gtaattataa atgaagatca agtatcaaaag ttaatagatt tagtattaca aaaaaataat 1380
gaaactcaag aatactctat aaataagaaa tgggtcggaa aagatgcaaa attattctta 1440
gatgaaatag atggttagtc tccttcaagt gttaaatgca taatctgca agtaagtgca 1500
aggcatccat ttgttatgac agaactcatg atgccaatat taccaattgt aagagttaa 1560
gatatagatg aagctattga atatgcaaaa atagcagaac aaaatagaaa acatagtgcc 1620
tatatttatt caaaaaatat agacaaccta aataggtttg aaagagaaat cgatactact 1680
atctttgtaa agaatgctaa atcttttgcc ggtgttggtt atgaagcaga aggctttaca 1740
actttcacta ttgctggatc cactggtgaa ggaataactt ctgcaagaaa tttacaaga 1800
caaagaagat gtgtactcgc cggttaataa ggctgagatc ttcttcagtg cattgtagtt 1860
gaatgaaggg ttagggggga aatgcccccc tattttttgt ctagccatcc tgccacgttt 1920
gacagggtag caatttcgac acgatagggt tctctcttct gccgtta 1967

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<210> SEQ ID NO 36

<211> LENGTH: 1602

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 36, Example
36: designer oxyphotobacterial Butyryl-CoA Dehydrogenase DNA
construct (1602 bp)

```

<400> SEQUENCE: 36

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agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctggcgctgc 60
aatcaccgca ttccccaacg cgctttgggc cacatgaccc ccatcgagag actccgaacg 120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat 180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa 240
aaaagtagca aaatttaca atgttcatga ttcatctggc taaattggat gttcaactga 300
cccattgaag acaagggcaa caaccatgaa tttccaatta actagagaac acaattagtt 360
acaacaaatg gttagagaat tcgcagtaaa tgaagttaag ccaatagctg ctgaaatcga 420
cgaacacagaa agattoccta tggaaaacgt tgaaaaaatg gctaagctta aatgatggg 480
tatcccattt tctaaagaat ttggtggagc aggcggagat gttctttcat atataatagc 540
tgtggaagaa ttatcaaaag tttgtgttac tacaggagtt attctttcag cgcatacatc 600
attatgtgca tcagtaatta atgaaaatgg aactaacgaa caaagagcaa aatatttacc 660
tgatctttgc agcggtaaaa agatcgggtgc tttcggatta actgaaccag gtgctgggtac 720
agatcgtgca ggacaacaaa caactgctgt attagaaggg gatcattatg tattaaatgg 780
ttcaaaaatc ttcataacaa atgggtggagt tgctgaaact ttcataatat ttgctatgac 840
agataagagt caaggaacaa aaggaatttc tgcattcata gtgaaaaagt cattcccagg 900
attctcaata ggaaaattag aaaataagat ggggatcaga gcactctcaa ctactgagtt 960

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agttatggaa aactgcatag taccaaaaga aaacctactt agcaaagaag gtaagggatt 1020
tggtatagca atgaaaactc ttgatggagg aagaattggt atagctgctc aagctttagg 1080
tattgcagaa ggagcttttg aagaagctgt taactatatg aaagaaagaa aacaatttgg 1140
taaaccatta tcagcattcc aaggattaca atggtatata gctgaaatgg atgttaaaat 1200
ccaagctgct aaatacttag tatacctagc tgcaacaaag aagcaagctg gtgagcctta 1260
ctcagtagat gctgcaagag ctaaattatt tgctgcagat gttgcaatgg aagttacaac 1320
taaagcagtt caaatctttg gtggatatgg ttactactaa gaataccag tagaaagaat 1380
gatgagagat gctaaaaat gcgaaatcta cgaaggaact tcagaagttc aaaagatggt 1440
tatcgcagga agcattttta gataaggctg agatcttctt cagtgcattg tagttgaatg 1500
aagggttagg ggggaaatgc cccctatatt tttgtctagc catcctgcca cgtttgacag 1560
ggtagcaatt tcgacacgat agggttctct cttctgcegt ta 1602

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<210> SEQ ID NO 37

<211> LENGTH: 1248

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 37, Example 37: designer oxyphotobacterial Crotonase DNA construct (1248 bp)

<400> SEQUENCE: 37

```

agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc 60
aatcacgcga ttccccaaag cgctttgggc cacatgaccc ccacgagag actccgaacg 120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat 180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa 240
aaaagtagca aaatttaca atgttcataa ttcattctgc taaattggat gttcaactga 300
cccattgaag acaagggcaa caaccatgga attaaaaat gttattcttg aaaagaagg 360
gcatttagct attgttaca tcaatagacc aaaggcatta aatgcattga attcagaaac 420
actaaaagat ttaaatgttg ttttagatga tttagaagca gacaacaatg tgtatgcagt 480
tatagttact ggtgctggtg agaaatcttt tggctgctga gcagatattt cagaaatgaa 540
agatcttaat gaagaacaag gtaaagaatt tggatattta ggaataatg tcttcagaag 600
attagaaaaa ttggataagc cagttatcgc agctatatca ggatttgctc ttggtggtgg 660
atgtgaactt gctatgtcat gtgacataag aatagcttca gttaaageta aatttggctc 720
accagaagca ggacttggaa taactccagg atttgggtga actcaaagat tagcaagaat 780
agttggacca ggaaaagcta aagaattaat ttatacttgt gaccttataa atgcagaaga 840
agcttataga ataggcttag ttaataaagt agttgaatta gaaaaatga tggaagaagc 900
aaaagcaatg gtaacaaga ttgcagctaa tgctccaaaa gcagttgcat attgtaaaga 960
tgctatagac agaggaatgc aagttgatat agatgcagct atattaatag aagcagaaga 1020
ctttgggaag tgctttgcaa cagaagatca aacagaagga atgactgcgt tcttagaaag 1080
aagagcagaa aagaattttc aaaataaata aggctgagat cttcttcagt gcattgtagt 1140
tgaatgaagg gttagggggg aaatgcccc ctattttttg tctagccatc ctgccacggt 1200
tgacagggta gcaatttcga cacgataggg ttctctcttc tgccgtta 1248

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<210> SEQ ID NO 38

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<211> LENGTH: 1311
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 38, Example
38: designer oxyphotobacterial 3-Hydroxybutyryl-CoA Dehydrogenase
DNA construct (1311 bp)

<400> SEQUENCE: 38

```
agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc      60
aatcaccgca ttccccaacg cgctttgggc cacatgaccc ccatcgagag actccgaacg     120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat     180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa     240
aaaagtagca aaatttcaaa atgttcatga ttcactctggc taaattggat gttcaactga     300
cccattgaag acaagggcaa caaccatgaa aaagatTTTT gtacttggag caggaactat     360
gggtgctggt atcgttcaag cattcgcgta aaaaggttgt gaggtaattg taagagacat     420
aaaggaagaa tttgttgaca gaggaatagc tggaatcact aaaggattag aaaagcaagt     480
tgctaaagga aaaatgtctg aagaagataa agaagctata ctttcaagaa tttcaggaac     540
aactgatatg aagttagctg ctgactgtga tttagtagtt gaagctgcaa tcgaaaacat     600
gaaaattaag aaggaatctt ttgctgagtt agatggaatt tgtaagccag aagcgatttt     660
agcttcaaac acttcatctt tatcaattac tgaagttgct tcagctacaa agagacctga     720
taaagttatc ggaatgcatt tctttaatcc agctccagta atgaagcttg ttgaaattat     780
taaaggaata gctacttctc aagaaacttt tgatgctggt aaggaattat cagttgctat     840
tggaaaagaa ccagtagaag ttgcagaagc tocaggattc gttgtaaacg gaatcttaat     900
ccaatgatt  aacgaagctt cttcatcctc tcaagaagga atagcttcag ttgaagatat     960
tgatacagct atgaaatag  gtgctaacca tccaatggga cctttagctt taggagatct    1020
tattggatta gatgtttgct tagctatcat ggatgtttta ttcactgaaa caggtgataa    1080
caagtacaga gctagcagca tattaagaaa atatgttaga gctggatggc ttggaagaaa    1140
atcaggaaaa ggattctatg attattctaa ataaggctga gatcttcttc agtgcattgt    1200
agttgaatga agggttaggg gggaaatgcc cccctatTTT ttgtctagcc atcctgccac    1260
gtttgacagg gtagcaattt cgacacgata gggttctctc tcttgcgctt a          1311
```

<210> SEQ ID NO 39
<211> LENGTH: 1665
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 39, Example
39: designer oxyphotobacterial Thiolase DNA construct (1665 bp)

<400> SEQUENCE: 39

```
agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc      60
aatcaccgca ttccccaacg cgctttgggc cacatgaccc ccatcgagag actccgaacg     120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat     180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa     240
aaaagtagca aaatttcaaa atgttcatga ttcactctggc taaattggat gttcaactga     300
cccattgaag acaagggcaa caaccatggg caaagaaagt agtttttagct gtgcatgtcg     360
```

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tacagccatc ggaacaatgg gtggatctct tagcacaatt cctgcagtag atttaggtgc	420
tatcggttate aaagaggctc ttaaccgcgc aggtgttaaa cctgaagatg ttgatcacgt	480
atacatggga tgcgttattc aggcaggaca gggacagaac gttgctcgtc aggettctat	540
caaggctggt cttcctgtag aagtacctgc agttacaact aacggtgtat gtggttcagg	600
tcttaactgt gttaaccagg cagctcagat gatcatggct ggagatgctg atatcgttgt	660
tgccggtggt atggaaaaca tgtcacttgc accatttgea cttcctaag gccggttacgg	720
atatcgtag atgtggccaa gccagagcca ggggtgctt gtagacacta tggtaagga	780
tgctctttgg gatgctttca atgattatca tatgatccag acagcagaca acatctgcac	840
agagtggggt cttacacgtg aagagctcga tgagtttgea gctaagagcc agaacaaggc	900
ttgtgcagca atcgaagctg gcgcattcaa ggatgagatc gttcctgtag agatcaagaa	960
gaagaaagag acagttatct tcgatacaga tgaaggccca agacaggggtg ttacacctga	1020
atctctttca agcttcgtc ctatcaacaa ggatggattc gttacagctg gtaacgcttc	1080
aggtatcaac gacggtgctg cagcactcgt agttatgtct gaagagaagg ctaaggagct	1140
cggcgtaag cctatggcta cattcgtagc tggagcactt gctgggttgc gtcctgaagt	1200
tatgggtatc ggtcctgtag cagctactca gaaggctatg aagaaggctg gtatcgagaa	1260
cgtagctgag ttcgatatca tcgaggctaa cgaagcattc gcagctcagt ctgtagcagt	1320
tgtaaggat cttggaatcg acgtccacaa gcagctcaat cctaacgggtg gtgctatcgc	1380
tcttgacac ccagttggag cttcaggtgc tcgtatcctt gttacacttc ttcacgagat	1440
gcagaagaaa gacgctaaga agggctctgc tacactttgc atcggtggtg gtatgggatg	1500
cgctactatc gttgagaagt acgaataagg ctgagatctt cttcagtgca ttgtagttga	1560
atgaagggtt aggggggaaa tgcccccta tttttgtct agccatcctg ccacgtttga	1620
cagggtagca atttcgacac gatagggttc tctcttctgc cgta	1665

<210> SEQ ID NO 40

<211> LENGTH: 4071

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 40, Example
40: designer oxyphotobacterial Pyruvate-Ferredoxin Oxidoreductase
DNA construct (4071 bp)

<400> SEQUENCE: 40

agaaaatctg gcaccacacc tgatctgcaa gagacgetca cgcgatacct ctgggcgtgc	60
aatcaccgca ttccccaacg cgctttgggc cacatgaccc ccacgagag actccgaacg	120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat	180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa	240
aaaagtagca aaatttcaaa atgttcatga ttcattctggc taaattggat gttcaactga	300
cccattgaag acaagggcaa caacatggc gcagaggtgc aaggagcccg tcgacggaac	360
gacagccacg acgcacgtgg cctacttcat gagcgacagc gcgttcatct tccccatcac	420
gcccagctcg gtcattgtcc aggtcgccca cgagtgttcc atgaacggcc gcaagaacgc	480
cttcggccag cccaagatgg tccgccagat gcagagcgag gctgggtctg ccggcgccct	540
gcacggcgcg ctcagcgagg gagcgtggc gacgacgttc acgagcagcc agggcctgct	600
gctcatgatc cccaacatgt acaagatcgc cggcgagctc ctgccctgcg tcatgcacat	660

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cgccgcccgc accgtcgcca ccgaggeect ctctatcttc ggcgaccaca cggatgtcta	720
cgcggtgagg tcgacggggg tgcggttccg gtgctccgcg accgtccagg agtgcaccca	780
catgtccgce gccgcgcacg ccgccacct gtccagcgag gtcccgttcg cccacttctt	840
cgacggcttc cgcacgtccc acgagatcca gaagatcgac tccccctcgg acgcccacct	900
gctggcctgc atgaactttg acgacgtccg caggttccgt ggccgctcgc tgtgctgcca	960
gcgcccgtg ctgcgcggga cggcgcagaa ccccgacgtc ttcacgcagg cgtccgagtc	1020
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caaggtgttc gggaccaact acaggaccta cgagtactat ggccaccccg aggccacgga	1140
ctgatcgtg gccatgggaa gcggcaccga agtggccatc tcgactgcca acttcctcaa	1200
ctcgcgcgac gcgaactcga gggctggcgt cgtgaggggt cggctgttcc ggcggtttgt	1260
gtcggcggcg tttgtggctg cgctgcccga gaccgtcaag aggatctgcg ttctggaccg	1320
cgggagggac gggcagggcg ccgcccagcc cctgcaccag gacgtcctgt cggcgctggg	1380
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gaaacaggtc agcgtcggca tcgctcgacga cgtgacgcac aacagcctgg acatgggaga	1560
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cgcgcagggg tactttcgct acgacgcca caaggccggc ggccctgacag tctcgcacct	1740
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gatcgccaag gacaacggga tgggcccgtt catcaacatg gtccctcagg ccgtgttctt	2040
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gaagatgtac gcgcgcaagg gcgaggaggt tgtcaggaag aacgtggcat cggtcgacgc	2160
gtcgtgggat cccaaggcgt tgctgcacat cgagtacccc gcagacaggt ggcttgcgct	2220
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gtgcccgcac gccctcatcc ggtcgtacca gatcagcgag gaggagatga agaacgcccc	2520
tgccgcttc gacactctta agtcgcgcaa gcccggttat cgtttccgca tcaacgtcag	2580
cgccctggac tgcactggct gcagcgtgtg cgtggagcag tgcccagtca agtgcctgga	2640
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cacgccgctg ttcgagttcc cgggagcctg cgcgggtgc ggtgagaccc cgtgggtgcg	2820
cctcgtgacg cagatgttcc gtgagcgcac ggtcatcgcc gcggccactg ggtgcaactc	2880
gatctgggga gcgtcgttcc cgaacgtgcc gtacacaacc aacgcccgcg gggaggccc	2940

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cgcgtaggcac aactcgtgtg tcgaggacgc ggcggagctc gggtagtgca ttacgtgtgc 3000
gtatcgccag cgcccgagc gcctcatcgg catcgtgcgg agcgtcgtcg acgatgcggg 3060
atcctgtcag ggtctgtctg ctgagctgaa ggctctgctg gtcgagtggc tcgcgcacgt 3120
cagggacttc gagaagaccc gcgagctccg cgacaggatg aaccccctga tcgacgcaat 3180
cccagcgaac gcggactgca gggttctgga gctcaggag aagcacaacc gcgagctgat 3240
cgcgcgacag agtttctgga tcctcggtag cgacgggtgg gcgtacgaca tcggcttcgg 3300
tggactggac cacgtgatcg ccaacaacga ggacgtcaac atccttgctc tcgacacgga 3360
ggtctactcc aacctggtg gccagcgtc caagtcgacg ccgctcggcg cccgcgccaa 3420
gtacgctgtg ctgggcaagg aacctgggaa gaaggacctg gggcgcatcg cgatgacctg 3480
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gacgggctac tggctgctgt accgcttcaa ccccgacctc atccacgagg gcaagaacct 3720
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ccgtttcatt actctgcagc gcgagcacc cgagcaggcc cacctccttc acgaggcact 3840
caccgctct ctggccacc gcttcgtgag ctaccagcgc ctctgtagc tgtacgagcc 3900
cgctgcccet gccgcagctc ctgccacgca ttaaggetga gatcttcttc agtgcattgt 3960
agttgaatga agggtaggg gggaaatgcc cccctatctt ttgtctagcc atcctgccac 4020
gtttgacag gtagcaatt cgacacgata gggttctctc ttctgcccgt a 4071

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<210> SEQ ID NO 41

<211> LENGTH: 1806

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 41, Example
 41: designer oxyphotobacterial Pyruvate Kinase DNA construct
 (1806 bp)

<400> SEQUENCE: 41

```

agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc 60
aatcaccgca ttcccacag cgctttgggc cacatgacct ccacgagag actccgaacg 120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat 180
agttagcaat aaatacagtt tcagaatata tgtaatacaa aaactgtatc gagacaagaa 240
aaaagtagca aaatttcaa atgttcatga ttcattctggc taaattggat gttcaactga 300
cccattgaag acaagggcaa caaccgtgtt cactaaaatt gtagctacat tggggccttc 360
gactgataga ctgccggata taacggccct gttgagcaag gttcacggcg tgcggataaa 420
tatgtctcac gcatcgccat cggaggtaga ggcccgcgtg aacgccgtga ggaagtatga 480
ggagaccagc gggaggtata tagccattat agcggatcta aggggcccga gcgtcaggac 540
cggccttatg cgcctctac agataacggc gggcgcccgc gtctccttta aattagccga 600
gaagggggac ggcttcgtac ctgtgccgag gcgtgagttc ttcgaagtaa tcgaggaggg 660
agacgaggtt ctatgttag acgaaaaact cgtcttgagg ataactcagc cagcgcagac 720
ctcggccgag gccgagctgt tatcctccgg cgtcatatcc agcaataagg caatagtggt 780
caaaggcaag gaatatcata tagagcagcc tgtggaggaa gacataaggg cgcttcagac 840

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gctctctcgg ttcagagacg acgtagacta cgtggccctc agccttgtga gagacggagc 900
agacgtgagg aaaatgagga gcgctcgcga ggaggctggg ctcacctccg gcataatggc 960
caaaatagag acgaagagcg cagtagataa aatcgaggag ataataatg cggccgacta 1020
catagttata gcgagaggcg atctggcgct gcactacgga ctggagtaca ttcctaaagt 1080
acagaggctc ttggtggaga gatctctctc ggcaggaagg cccgtggcgg tggccacgca 1140
gcttttggac tctatgcaga ccaacacgac gccactagg gcggaggtea acgacgtgta 1200
cacaacggcg agtctcggag tggactctct gtggctgacc aacgagactg cgagcggaga 1260
gcacccgta gaggcagtgg attggctgag gaggatagtg tcgaggtcg agttcgggag 1320
acttaaggct gcgctgccgg ccgacgcacg cgataggttc gccaaagccg tggtagatat 1380
ggccgaggac atgggagggg aaatcgacgt atactcaatg acgggaactc tggcgaagag 1440
aatagctaaa tttagccga tgacgacagt ctacgtcggg gtcaacgaga ggaggctcgc 1500
gaggatggtg gagctccgcg aggatggttg agctcatatg gggcctagag cctgtggtcg 1560
tgccggcgca tacttacgag gagggctcag agaggctcct ctccagattc tccgacaaag 1620
tcttgatagc cacgtatggg ctcagaggcg gcacacatac tattaataag gctgagatct 1680
tcttcagtgc attgtagttg aatgaagggt taggggggaa atgccccct atttttgtc 1740
tagccatcct gccacgtttg acagggtagc aatttcgaca cgatagggtt ctctctctg 1800
ccgtta 1806

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<210> SEQ ID NO 42

<211> LENGTH: 1696

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 42, Example 42: designer oxyphotobacterial Enolase DNA construct (1696 bp)

<400> SEQUENCE: 42

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac cttatttttt cttcaagtta aagaatgcgt tcattccagg ataaacggca 300
atgctgccaa gctcttcttc aattctcaag agctgattgt attttgetac tctgtctggt 360
cttgacggtg cacctgtctt tatctgacca gcatttactg caacaacaag gtcagcaatt 420
gttgatctt cagtctcacc tgatctgtgg gatacaactg cagtgtagcc tgetctatct 480
gccatttcaa tagcttctaa agtttctgta agtgttctta tctgattaag cttaatcaat 540
attgagtttg caacgccaag ttctattccc tttgcaagcc tctttgtggt tgtaacaaac 600
aaatcatcac ccacaagctg aatcttcttg ccaagtgett cagttagcat cttccagcct 660
tcccagtcct cttctgcaac accgtcttca attgatacaa ttgggtactt ttcaacaagt 720
tttaccaga attctaccat ttcttctttt gttctaactt taccttctct ttcgaaatga 780
tactttccat cttcttcatt gtagagctca gatgttgca ggccaagcgc aattgcaata 840
tccttaccag gagtataacc agctttttca attgcttcga caattacttc caatggctct 900
tcgttagact tcaagtttgg tgcaaatcca ccttcacac ccactgttgt gttgtatcct 960

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cttgccttca atacatttct taattgatgg aatgtctcag cacacatcct gagtgtctcg 1020
ctaaaagatt ttgcaccaac tggcattatc ataaactctt gtaggtcaac agagttgtca 1080
gcatgcttcc caccgttcaa aatattcatc attggcacag gtaaaacttt tgcattgaca 1140
ccaccaatgt attggtacag tgggaagacca agtgcggttg ccgctgcctt cgcaactgcc 1200
aaagatacac ccaaaattgc atttgacca agcttgctct tgttctctgt cccatcaagc 1260
tcaatcataa gcctgtcaat ctcaacttgg ttaagagcgt tcattccaat tattttctggc 1320
gcaataacct cgtttacatt ttcgactgct ttgagaaccc cttttcccat atatcttttt 1380
ttatcaccgt ctctgagttc aacagcctcg aacatacctg ttgacgcacc tgatggaaca 1440
gcagctctac ctacaaatc atcatttaca acaactteta cttcaacagt tgggtttcct 1500
cttgaatcca gaatttctct tgcttttaca gctgtaattg aaagatcaac cttcattaag 1560
gctgagatct tcttcagtgc attgtagttg aatgaagggt taggggggaa atgccccct 1620
atttttgtc tagccatcct gccacgtttg acagggtagc aatttcgaca cgatagggtt 1680
ctctcttctg cgtta 1696

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<210> SEQ ID NO 43

<211> LENGTH: 2029

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 43, Example
43: designer oxyphotobacterial Phosphoglycerate-Mutase DNA
construct (2029 bp)

```

<400> SEQUENCE: 43

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaaatg tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac cctacgccgg cgcctgcttc tctcctctgc ggcagcactt ctccaaaggg 300
tgcacgttcg cttctctgtg aatcagggtc tggccgggtc tctcggccgg ttcgggatg 360
cccagcaggt gcaggatggt gggggccaca tcccgcaggc tgcctgccg cagcgcagt 420
ccggcgggat cccgcccgat caggatgaac ggcaccgggc tgggtggtgtg ggcgatga 480
ggctgtccct cttcgtccac catctcatcc gcattgccgt ggtctgccgt taccaggagc 540
gtgccgtcct tttccaggac ggcccgcgcc acctttccaa ggcagcggtc gattgtttct 600
atggccttta ccgttgcctt catgtgcggg gtatgcccca ccatgtcggg attggcgtaa 660
ttcattatga ttacgtcgta cttgcccag gccagccgct ccagaaaggt gccggtgacc 720
tcggtggcgc tcatttcggg cttcaggtcg taggtggcca cccgcgggga gggcaccagg 780
atcctgtcct cgcgggggta tggcttttct aagccgccgt tgaagaagaa ggtcacatgg 840
gcgtactttt ccgtttcgca caggcgggag tgggtcatgc cgtgcctgct taaaacctcg 900
cccagggtat tgcgcagctc ctgcggctga aacgccaccg gcgccttaat ggtcttctcg 960
taaagggtca tgcaggtaaa atgcaaggca gggtagccct gctttctggc aaacccggtg 1020
aaatcctcgt ccacaaggc cctggtaatc tggcgggccc ggtccgccc gaagttaaag 1080
aaaaaacg cgtegccctt cattattttg gcggccggcc caccgaccc gtttaccacg 1140

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acgggtgggct ggataaactc gtcgggtttca tcccttccgt accccaggtc aaccgctccc 1200
agcgggcttg ttgcctgaat gccctcgctt aaaaccattg cgttgtaacg ccgctcggtg 1260
cgggtcccagc ggcggtctct gtccatggcg taatagcgcc ccattaccgt tgcccacgcc 1320
ccaaagccca gttcgcccag cttcttcctt aactgctcga agtattcttt tgcggtggcc 1380
ggcggcaccg cgcgcccgtc caggaaggca tggacaaaaga cgttgcgcat gttctcggg 1440
gcbggccagg ccaggaggcc gaaaaggctg ctgatatggc tgtgcaactc gccgtccgat 1500
aaaagcccca tcagggtgaag ggccttatta ttctccctgg cgtatctcac cgctccagc 1560
aggacttcgt tcttgaaaaa ggtcccgtcc ttgatggcgc ggcttattct ggtaagctcc 1620
tggtacacca ccctgcccgc gcctatgttc aagtgtccca cctcggaatt gcccatctgg 1680
ccctcgggaa gccccacgtc ctcgcccga cagctcaggg cacagtgggg gtaaccggcc 1740
agaaagctct tgaatttcgg tgtgtcggcc agggctatgg cattgcccgc gacattggaa 1800
ctgagggccc agcctccagc aaccaccagc accaggggccc tgcgcccggc ataccggccc 1860
cagggcggtg cagctacgtc ttccttcaat aaggctgaga tcttcttcag tgcattgtag 1920
ttgatgaag ggttaggggg gaaatgcccc cctatttttt gtctagccat cctgccacgt 1980
ttgacagggc agcaatttcg acacgatagg gttctctctt ctgccgta 2029

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<210> SEQ ID NO 44

<211> LENGTH: 1687

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 44, Example
44: designer oxyphotobacterial Phosphoglycerate Kinase DNA
construct (1687 bp)

```

<400> SEQUENCE: 44

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agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcccggc cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac cttatttate gagcagcgcc cttactcccg gcagttgctt cccttccaga 300
aactccaggg aagcgcggcc gccggttgag atatgggtca ttttcccggc tacgccggcc 360
ttcttggecg ccgcccggct gtcaccggcc cggattacgg tgacggcggt taattcggcc 420
agcgtccggg ctattgcttc ggtgcccctg gcaaaaggat ccatttcaaa aacgccatt 480
ggtccgttcc agaccacggt cctggcccgc ctgagggcct cggtgaaaag tctgatggac 540
tcgggcctta tatccagggc catccactcc gccgggattt gatcgaccgg caccgtcctt 600
tgctcctgge cgggcgcccg ccccggcgcc accaccacat ccaccggcag gaggagcttt 660
acttccctgc ttctggcttc tgcaatcagc ttcctggcca ggtcaatctt gtcggcctcc 720
agcagggact taccgacgct gtacccttgt gccttcagaa aggtattggc catcccgcg 780
ccaatgataa ccgatcagc tttggtcagc aggttgaaaa ttactcccag cttgtcggaa 840
actttcgagc cgcaccagc ggctgcaaaa gggcgctccg ggctggctcag cagcctgccc 900
agtatttcca gctcttttcc catcagcagg cctgccacgg ccggcaaaaa cccggcaacg 960
ccctcggtag aggcgtgggc ccggtgtgcy gttccaaacg catcgtttac aaagacatct 1020
gccagctcag ccagttgcg ggcacacttc tcgtcgtttt tctcctctc cgggtggaaa 1080

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cggacgtttt ccagcagcac cacgtcccgc tctgcatct gggcaacggc ggacctggcg 1140
gcttctccca cgcagtcgcc ggccttaacc accgttttcc ccagcagttc ggaaggcg 1200
ctggcaacgg gatccatttt gtacctctcg tccacctgc ccttggggcg gccaggtgc 1260
gaaaccagaa taacctggc tttttgtccg ataaggtagt ttatggtggg cacggcctcc 1320
tttattttaa cgtcatcgcc caccggcgcc ttttccatcg gcacgttgaa gtccaccgc 1380
aacaggacce gcttgcctt tacatctata tcccttacg ttttttggc cactaaggt 1440
gagatcttct tcagtgcatt gtagtgaat gaagggttag gggggaaatg cccccctatt 1500
ttttgtctag ccatcctgcc acgtttgaca gggtagcaat ttcgacacga tagcgtgctg 1560
tactgttttt tgctcgtcag ggttgggttt tgctcatgac acccaaggat tggagtcggt 1620
gctcaataat cgcagttgc tgttgggcag ccgccaattg cgctgaggt tctctcttct 1680
gccgtta 1687

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<210> SEQ ID NO 45

<211> LENGTH: 1514

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 45, Example:
designer oxyphotobacterial Glyceraldehyde-3-Phosphate
Dehydrogenase DNA construct (1514 bp)

```

<400> SEQUENCE: 45

```

agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc 60
aatcaccgca ttcccacacg cgctttgggc cacatgacct ccacgagag actccgaacg 120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat 180
agttagcaat aaatacagtt tcagaatctc tgtaatacaa aaactgtatc gagacaagaa 240
aaaagtagca aaatttcaa atgttcatga ttcattctggc taaattggat gttcaactga 300
cccattgaag acaagggcaa caaccaatgg atttgggcgg atcggacggt tagcattcag 360
aagaattcaa gatgtagaag gtcttgaagt agttgcagtt aacgacttaa cagatgacga 420
tatgttagct catttattaa aatacgtatc tatgcaaggt cgtttcactg gagaagttga 480
agttatcgaa ggtgattcc gtgttaacgt aaaagaaatt aaatcattcg atgaccagat 540
gctgggtaaa ttaccatggg gcgatttaga tatcgacgta gtattagaat gtactggttt 600
ctatactgat aaagaaaaag cacaagctca catcgatgca ggtgctaaaa aagtattaat 660
ctcagctcca gctaaagggt atgtaaaaac aatcgtattc aacactaacc atgacgcatt 720
agacggttca gaaacagttg tttcaggtgc ttctgtact actaactcat tagcaccagt 780
tgcaaaaagt ttaagtgatg aattcgggtt agttgaaggt ttcattgact caattcacgc 840
ttacactggt gacaaaaata cacaagacgc acctcacaga aaaggtagca aacgtcgtgc 900
acgtgcagca gcagaaaata ttatccctaa ctcaacaggt gctgctaaag ctatcggtaa 960
agttattcca gaaatcgatg gtaaattaga cgggtggagca caacgtgttc cagttgctac 1020
tgggtcttta actgaattaa ctgtagtatt agacaaaaca gatgtaactg ttgaccaagt 1080
taacagtgct atgaaacaag cttcagacga atcattcgggt tacactgaag acgaaatcgt 1140
atctctgat atcgttggtg tgacttacgg ttcattattc gatgcgactc aaactcgtgt 1200
tatgactggt ggagatcgtc aattagttaa agttgcagct tggtagcaca aagagtgggg 1260

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taaggctgag atcttcttca gtgcattgta gttgaatgaa gggttagggg ggaatgccc 1320
ccctatTTTT tgtctagcca tctgcccag tttgacaggg tagcaatttc gacacgatag 1380
cgtgctgtac tgttttttgc tcgtcagggg tgggttttgt catcgacacc caaggattgg 1440
agtcggtgct caataatcgc cagttgctgt tgggcagccg ccaattgcgc ctgaggttct 1500
ctcttctgcc gtta 1514

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<210> SEQ ID NO 46
<211> LENGTH: 609
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 46, Example
46: designer Nial-promoter-controlled Proton-Channel DNA construct
(609 bp)

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<400> SEQUENCE: 46

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agaaaatctg gcaccacacc tatatggtag ggtgagagtg accccgcgcg acttgagct 60
cgatggcccc gggttgtttg gggcgteccg ctctcgcgct attctgagct ggagaccgag 120
gcgcatgaaa atgcattcgc ttccatagga cgctgcattg tggettgaag gttcaaggga 180
agggttcaaa cgaccccgcc gtacgaactt ttgtcggggg gcgctcccg ccccgggctc 240
ttgtgcgcg attagggctt cgggtcgcaa gcaagacgat acatggccgg catcgcgccc 300
gtgctgaagg tcctgaccac cggcctgccc gccctgatca gctggatcaa gcgcaagcgc 360
cagcagtaaa tggaggcgct cgttgatctg agccttgccc cctgacgaac ggcggtgat 420
ggaagatact gctctcaagt gctgaagcgg tagcttagct ccccgtttcg tgctgatcag 480
tctttttcaa cacgtaaaaa ggggaggagt tttgcaattt tgttggttgt aacgatcctc 540
cgttgatttt ggctcttctc tccatggcg ggctggcgct atttgaagcg gttctctctt 600
ctgccgtta 609

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<210> SEQ ID NO 47
<211> LENGTH: 1360
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 47, Example
47: designer nirA-promoter-controlled NAD-dependent
Glyceraldehyde-3-Phosphate-Dehydrogenase DNA construct (1360 bp)

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```

<400> SEQUENCE: 47

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```

agaaaatctg gcaccacacc cttcttgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcaatc gagaaactgc taatctgcc agtatatgaa cggatttggc 120
aggataggac gactggtgtt gcgggcgcg gtggagaagg gcacggtgga ggtggtggcg 180
gtgaacgate cgttcatctt cccggacgcg gcgtacgctg cgtacatgct gcagtacgac 240
tcgacgcacg gggcgttccc gggtaggtg ggcagcgacg gggagcactt ggtggtgaac 300
gggaagaagc tggcgtgctt tgcgatccgc gatccggcgg agatcccgtg gggctcggtc 360
ggcgccgact acgtcgtgga gtccaccggc gtgttcaccg tgaccgagaa ggcgtcgttg 420
cacgtcaagg gcgcgcgcaa gaaggtggtt atatcgccgc cgtcgaagga tgcgccatg 480
tttgtgatgg gcgtgaacca tgacgcctac accaaggact tgacggtggt gtcgaatgcg 540
tcttgcacca ccaacttgtt tggcgccgct ggccaagatc atcgacgagg cgttcggcat 600
cgggatggcg ctcatgagca ccatccacgc ggtgacggcc acgcaaaaga cggtaggatg 660

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gccgagctcc aaagactggc gcggtgtcgc ggcgcgttcc agtcgattat tcccagcagc 720
accggcgctg cgaagcgggt cggcaaggtg taccggaagc tgaacggcaa gctgaccggc 780
atggcgcttc gcgtgcccgt gcccgacgtg tccgtggtag acttgacagt gaccctgaag 840
aaggagacca actacgagga gatcaaaaag gctgtcaagc aggcgtcgcg gagcccgcac 900
tacaagggca tcgtggcgta caccgagcac cccatcgtgt cggccgacct ggtgcacaac 960
ccgtactcgt cgggtgtcga tgccgaagcc ggtatcatgc tgtcgcccac gtttgtgaaa 1020
ctggtcagct ggtaatagtg atcccggccg ctactaaagc ctgatttgc ttagatagctg 1080
ctctgcctt tgggcagggg cttttttctg tctgccattc ttgaggatgg cggactcttt 1140
cccttttget ctacgcccac gaatgcgacg gcagtcctcc ctgtccagca cgttggagtg 1200
attggtggtg gccagtttagc ttggatgctg gcaccagcag cgcaacagtt ggggatgtcg 1260
ctgcacgttc aaacacccaa tgatcacgac ccagcagtag cgatcgcgga tcaaacgta 1320
ttagcagcag ttgctgacgc ggttctctct tctgcccgtta 1360

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<210> SEQ ID NO 48

<211> LENGTH: 1621

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 48, Example
48: designer nirA-promoter-controlled Phosphoglycerate-Kinase DNA
construct (1621 bp)

```

<400> SEQUENCE: 48

```

agaaaatctg gcaccacacc cttctgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcgaatc gagaactgcc taatctgccg agtatatgtc atttgtcttc 120
gagcgcgacg acaccggcca gctgttttcc ttccataaac tcgagcgaag cgcgcgcgcc 180
ggtggagata tgatccattt tgctcggcaa gccgaatttc tcaaccgccg ccgccgaatc 240
cccgcgcgcg atgaccgaat aggtgtcggg cgcttcgccg agtgcttcgg cgatcgcttt 300
tgtccatgg gcgaacgctt ccatttcaaa gacgcccacg gggccgttcc agacaacgag 360
cttcgattga cgaatgacat cgcggtacaa ttcgcgcggt ttcgggccga tgtcaagcgc 420
ctcccaatcg ctccggaatg cgtcgatggc gacgacttcc gtgttgccgt cgttcgcaaa 480
ccggtcggcg acgaccacgt ccaccggcat ataaaaacgg acgccttttt ctttcgcctt 540
ttccataaac gattttggcg gttcgatttt gtcctcctca agcagcgact tgccgacgtc 600
atggccgagc gctttgacga acgtatacgc cagtccgccg ccgatgatca agttgtcgac 660
tttttcaage aaattgtcga tgacgccgat tttgtcttcc actttcgcgc cgcgatgat 720
cgcgtaaac gggcggctcc gattcgagag cgctttgccg agcacttcga gttctttttc 780
catcaaaaac ccggccaccg caggcaagta atggcgatg ccttcgctcg acgcatgagc 840
gcggtggggc gcgccgaacg catcgttgac atacagatcc gcgagctccg caaacgcttt 900
ggccagctct ggatcgtttt tctcttcgcc agggtaaaaa cggacgttct caagcaagag 960
cacgtegcct tcgttcaaac ggtcgaccgc cgcttccacc tcctcgcgga ccgcttcatt 1020
cgttttggcg accggccggt caagcagctc gccgagccgc ttcgcaacgg catccaaacg 1080
caattcttcg accacttttc ctttcggggc gccgaggtgg ctgcgcaaaa tgactttcgc 1140
cccgtgctcg atcaaatagc ggatcgtcgg gagtgccggc cgaatgcgcg tgtcatcggt 1200

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gatggcgcct tgctccatcg gaacggtgaa atcgacgcgg caaaagacgc gctttcccct 1260
cacctcaacg tcgcgatcg tcttctgtt cattaatagt gatcccgcc gctactaaag 1320
cctgatttgt cttgatagct gctcctgcct ttgggcaggg gctttttct gtctgccatt 1380
cttgaggatg gcgactcct tcccttttg tctacgccc tgaatgcgat cgcagtctcc 1440
cctgtccagc acgttggagt gattggtggt ggccagttag cttggatgct ggcaccagca 1500
gcgcaacagt tggggatgtc gctgcacgtt caaacaccca atgatcacga cccagcagta 1560
gcgatcgcg atcaaacctg attagcagca gttgctgacg cggttctctc ttctgccgtt 1620
a 1621

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<210> SEQ ID NO 49
<211> LENGTH: 1990
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 49, Example
49: designer nirA-promoter-controlled Phosphoglycerate-Mutase DNA
construct (1990 bp)

```

```

<400> SEQUENCE: 49
agaaaatctg gcaccacacc cttctgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcgaate gagaactgcc taatctgccg agtatatgct cgagcattta 120
ttaaataata agcgagcttc ccttcctctc tgaaggtttt tctaacccta agatgtctaa 180
gattgttggg caatgtctct ctaagattcc atcatctctt aatttaacat tgccatattc 240
cacaagatac aaaggcacct tatttgtgt atgagctgta tgaggctcac ctgtctcata 300
atcaatcacc tgttcacagt tgccatggtc agcagtaata ataaccactc cacccttttc 360
taaaccttg ttaacaactt ttccaataca ctcatctaca gcctcaactg cctttattgc 420
agcctctaaa acgcctgtgt gccctaccat gtcaccattt gcatagttac atattatcac 480
atcatattca tctctttcaa ttctctcaag taaagcttct gttacctcgt atgcactcat 540
ctcaggttta agatcatatg ttgcaacctt tgggtgatgg accaataacc tgtcttctcc 600
gacatttggg acttccacac cgccggtgaa gaaaaagggtg acatgagcat acttttctgt 660
ctcagcaatt cgaagtgtgt ttaaccctaa cttgctaaaa tactctccca aagtgtttgt 720
caggttctct ggtttgaatg caacatggca attttttatt gtcacatcat actgagtcac 780
gcatacaaa gaaacttcga aatatacctt tttcctttca aaaccgtcaa attcaacatc 840
acaaaaacgt cttgtaagct gtcttgcctc gtcaggtctg aagttaaaga aaataatact 900
gtcatgttca tttattgttg cgacaggttt tccattttca agcacaacag tcggaattac 960
aaactcatca gtgttacctt ttttatacga cttttcaacc gcctctaata ctgagcttgc 1020
atactcgcct tcaccaaaaga ccattgcatt atatgccttt tcaactcttt cccatctttt 1080
gtctctgtcc attgcatagt atctgcccac cactgttgca atcttaccac aaccaatttc 1140
ttttatcttc tgttcaagct cttcaatgta aatttttgcg ctggaagggtg gaacatctcg 1200
cccatccaaa aagcaatgaa catatacttt ttcaagattg tgccctcttg caagttttta 1260
aagtgcgtaa agatgtgtgt tgtggctgtg aacaccacca tctgataaaa gtcccacag 1320
atgaagagaa gagttatatt ttttgcaatt ctctattgcc atcaaaaact cttctttttc 1380
aaaaaaaaa ccgtctttta ttgactttgt tattcttgta aattcttggg aaacaattct 1440
tcttgcaccc aggttcagat gtccaacttc agaattcccc atttgtcctt cggaagacc 1500

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aacatccata ccactgctac caatcagggt atatgggtaa ttcttttctg aatagtcaag 1560
gtagggggtc ttacccaaag caacagcgtt tocctcttgc tttgggttat aaccccaacc 1620
gtccatgata atcaacacaa caggtttttt cattaateta gataatagtg atccccgccc 1680
ctactaaagc ctgatttgtc ttgatagctg ctctgcctt tgggcagggg cttttttctg 1740
tctgccattc ttgaggatgg cggactcttt cccttttctg ctacgcccac gaatgcatc 1800
gcagtctccc ctgtccagca cgttggagtg attggtggtg gccagttagc ttggatgctg 1860
gcaccagcag cgcaacagtt ggggatgtcg ctgcacgttc aaacacccaa tgatcacgac 1920
ccagcagtag cgatcgcgga tcaaaccgta ttagcagcag ttgctgacgc ggttctctct 1980
tctgccgtta 1990

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<210> SEQ ID NO 50

<211> LENGTH: 1765

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 50, Example
50: designer nirA-promoter-controlled Enolase DNA construct
(1765 bp)

```

<400> SEQUENCE: 50

```

agaaaatctg gcaccacacc cttctgacg aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcaatc gagaactgcc taatctgccg agtatatgct cgagcatatg 120
ctaaataaac ccgctgtttc cattgaagaa attaccgcta gagaaatfff agactctcgt 180
ggccgtccta ccattgaagc agaagtctta ctggaaacag gggctttcgg tattgcccag 240
gttcccagtg gcgcgtcaac tggtagcttc gaggccacg aattacggga tgatgacccc 300
aacccgtacg gtggtaaagg cgttctcaaa gcggttagta acggtataga cgaattgcc 360
cctaaaatta tcggaatgga tgggttagat caaactgcga tcgatcacac catgattgag 420
ttagacgggt ctactaataa aaaagaatta ggggccaatg ctatccttgc cgtttcctta 480
gccactgcaa aagctgccgc cgatgaatta gcccttcccc tgtaccgta tttaggggggt 540
cccttgcca atgtcttacc cgtccccatg atgaactgta ttaacggggg ttctcacgcg 600
gataataaac tagacttcca ggagtttatg attatgccag tgggtgcgga ctcttttaaa 660
gaagcttga ggtggggggc cgaagtgttt gcttccctca gtaaagtctt aaaagagcgt 720
aaattgctct ctggggtagg agacgagggg ggatcacccc cgaacctggg atcgaaccag 780
gaagccttag atttgcctat agaagccatt gaaaaggcgg ggtataagcc aggggaacag 840
gtggctttag cgatggatgt ggcttcaagt gagttttata aggatggcga atatatttat 900
gatggttctc cccattcccc tcaagaatff atcgattatt taggtaaatt agtggatcaa 960
tctcctatta tttccattga agatggctta caagaagatg actgggatag ctggaaaagt 1020
ttgaccgata cgtaggatc tcgcattcag ttagttgggg acgatctttt tgtcacgaac 1080
cccactgcgc tgcaaaaagg cattgatatg ggtgtgggta atagtattct cattaaactc 1140
aatcaaatg gtagtttaac ggaaacgta gatacgattg ctttagcgac tcgtcatcaa 1200
tatagttccg ttatttccca tcgttccgga gaaaccgaag aactaccat tgcagactta 1260
gccgtagcta cacgcgctgg acaaatcaaa accggttctc tgtgtcgtag tgaacgggta 1320
gccaaatata accgactatt acgtattgaa gaagaattag gcgatcgcgc agtttatgct 1380

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gcaaaagtgg gtttaggccc tcaataaggc tgctgccccg gctgctgcta atctagataa 1440
tagtgatccc ggccgctact aaagcctgat ttgtcttgat agctgctcct gcctttgggc 1500
aggggctttt ttctgtctgc cattcttgag gatggcggac tctttccctt ttgctctacg 1560
cccatgaatg cgatcgcagt ctcccctgtc cagcacgttg gagtgattgg tggtggccag 1620
ttagcttga tgctggcacc agcagcgcga cagttgggga tgctgctgca cgttcaaaaca 1680
cccaatgatc acgaccgcag agtagcgatc gcggatcaaa ccgtattagc agcagttgct 1740
gacgcgggtc tctcttctgc cgtaa 1765

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```

<210> SEQ ID NO 51
<211> LENGTH: 1888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 51, Example
51: designer nirA-promoter-controlled Pyruvate-Kinase DNA
construct (1888 bp)

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<400> SEQUENCE: 51

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agaaaatctg gcaccacacc cttcttgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcaatc gagaactgcc taatctgccg agtatatgct cgagcatatg 120
ttaaaaaaga cgaaaatcgt ttgcacgcag ggtccgtcca cagagaaaacc gggcgtaatt 180
gatgcactga ttgccaatgg catgaactgc gcacgcttca atttctccca tggtgaccac 240
gaagaacatc ttggccgat caatatggtt cgtgaagctg ccaagaaggc tggcaagggt 300
atctctttaa tctctgatac caaaggctcc gaaatgcgtc tggcgagtt caaagatggc 360
aaagttatgc tcgaaaaggg caacaagttc actttgacct atgacgatga accgggtgat 420
gaaactcatg tttccgtaaa ccacaagggt ctttacacgg aagttaagcc gggcgacacc 480
ctgctcctct ccgatggcct cgtagctctc aaagttgatg aaatcaaggc caaggatatac 540
gttacgacga ttcagaacag cggttaagatg agcacgcgca agcgcgtagc tgctccgggc 600
gtaccccttg gtctgcctcc tatctccgaa caggatgcta aggacatcat ctttggtctg 660
gaacaggata tggatttctg agctgcttcc ttcacccagc gtccggatga tgttatcgcc 720
atccgcaagc tcatcgaaga gcacaatggc cacatggaaa ttctgccgaa gatcgaaaac 780
ctcgaagggt ttaagaactc cgatgcaatc ctggaagttt ccgacggcat catggttgcc 840
cgtggtgacc tggcgtaga agttccggca gaagatgtgc cccttattca gaaggaaatc 900
atccgcaagt gcaacgctgc tggcaagccg gttatcgttg ctacgcagat gctcgactcc 960
atggaacgca accccgctcc gacccgtgca gaagtttctg acgttggtaa cgccatcctc 1020
gatggtacgg atgccatcat gctgtccggc gaaaacggctt ccggtgacta tccggtagaa 1080
gcagttgcca cgatgaaccg cattgcacag cgcattgaaa gctcccttga atacaaggaa 1140
ctctatgtag aacgtggtct gcagcacatg gaatcccgta cgcgtgctat cgctcatgct 1200
acggttcaga tggcttatga gctcgatgct ccgctatta tcacgcccag cgaatccggg 1260
tacacgacga aggtcgtttc caagtatcgt ccgaaggctg ctatcgtagc ttacacgccg 1320
agcgaaaaag ttctgcgtca gctgaacctg cgttggggcg tataatccggg actcggcacc 1380
cagtgagcgc atgtggatga aatgatcagc aatgcaacgg ctgctgctgt taaggaagac 1440
ctcgtacagc gcggcgacct caccatcatc acctccggtg tgaagatgga atcccgtagc 1500
cgtgctatcg ctcatgctac ggacatctaa ggctgctgcc ccggtgctg ctaacttaga 1560

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taatagtgat cccggccgct actaaagcct gatttgtcct gatagctgct cctgcctttg 1620
ggcaggggct tttttctgtc tgccattcct gaggatggcg gactccttcc cttttgctct 1680
acgcccataga atgcgacgac agtctccctc gtccagcagc ttggagtgat tgggtggtggc 1740
cagtttagctt ggatgctggc accagcagcg caacagttgg ggatgctgct gcacgttcaa 1800
acaccaaatg atcacgaccc agcagtagcg atcgcggatc aaaccgtatt agcagcagtt 1860
gctgacgagg ttctctcttc tgccgtta 1888

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<210> SEQ ID NO 52

<211> LENGTH: 2188

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 52, Example
52: designer nirA-promoter-controlled Pyruvate-Decarboxylase DNA
construct (2188 bp)

```

<400> SEQUENCE: 52

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agaaaatctg gcaccacacc cttctgacg aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcaaatc gagaactgcc taatctgccg agtatatgct cgagcatatg 120
gtatcaacct acccagaatc agaggttact ctagggaagg acctccttga ggcactccac 180
caattgaaa tgacacccat tttcggcttg cgggtgact tcaaccttc cttattggac 240
aaagtgtatg aagttccgga tatgaggtgg gctggaaatg ccaacgaatt gaatgctgcc 300
tatgtgccc atggttactc cagaataaag ggattgtcct gcttggtcac aacttttgg 360
gttgggtaat tgtctgcttt aaacggagtt ggtggtgcct atgctgaaca cgtaggactt 420
ctacatgctg ttggagtctc atccatctcgc tcacaggcta aacagttggt gctccaccat 480
accttgggta atggtgactt cactgttttt cacagaatgt ccaatagcat ttctcaaaact 540
acagcatttc tctcagatat ctctattgca ccagggtcaa tagatagatg catcagagaa 600
gcatatgttc atcagagacc agtttatggt gggttaccgg caaatatggt tgatctcaag 660
gttctctcta gtctcttaga aactccaatt gatttgaat tgaacaaaa tgatcctgaa 720
gctcaggaag aagttgttga aacagtcctg aagttggtgt cccaagctac aaacccatt 780
atcttgtag acgcttgtgc cctcagacac aattgcaaag aggaagtcaa acaattgggt 840
gatgccacta attttcaagt ctttacaact ccaatgggta aatctggtat ctccgaatct 900
catccaagat ttggcgggtg ctatgtcggg acaatgtcga gtcctcaagt caaaaagcc 960
gttgaaaatg cggatcttat actatctggt gggtcgttgt tatcggactt caatacaggt 1020
tcattttcat actcctacaa gacgaagaat gttgttgaat tccactctga ctatatgaaa 1080
atcagacagg ccaccttccc aggagttcaa atgaaagaag ccttgcaaca gttgataaaa 1140
agggctctct cttacatcaa tccaagctac attcctactc gagttcctaa aaggaaacag 1200
ccattgaaag ctccatcaga agctcctttg acccaagaat atttgtggtc taaagtatcc 1260
ggctggttta gagaggggta tattatcgta accgaaactg gtacatctgc tttcggaatt 1320
attcaatccc attttcccag caacactatc ggtatatccc aagtctgtg gggctcaatt 1380
ggtttcacag taggtgcaac agttggtgct gccatggcag cccaggaaat cgaccctagc 1440
aggagagtaa tttgttctg cggtgatggt tcattgcagt tgacgggtca ggaatctct 1500
acgttgtgta aatgggattg taacaatact tatctttacg tgttgaacaa tgatggttac 1560

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actatagaaa ggttgatcca cggcaaaagt gccagctaca acgatataca gccttggaac 1620
catttatcct tgcctcgctt attcaatgct aagaaatacc aaaatgtcag agtatcgact 1680
gctggagaat tggactcttt gttctctgat aagaaatttg cttctccaga taggataaga 1740
atgattgagg tgatgttate gagattggat gcaccagcaa atcttgttgc tcaagcaaag 1800
ttgtctgaac gggtaaacct tgaaaattga ggctgctgcc cgggctgctg ctaactctaga 1860
taatagtgat cccggccgct actaaagcct gatttgtcct gatagctgct cctgcctttg 1920
ggcaggggct tttttctgtc tgccattcct gaggatggcg gactcttcc cttttgctct 1980
acgccatga atgcgatcgc agtctccct gtccagcagc ttggagtgat tgggtgtggc 2040
cagttagctt ggatgctggc accagcagcg caacagttgg ggatgtcgtc gcaagttcaa 2100
acaccaatg atcacgaccc agcagtagcg atcgcggatc aaaccgtatt agcagcagtt 2160
gctgacgagg ttctctcttc tgccgtta 2188

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<210> SEQ ID NO 53

<211> LENGTH: 1510

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 53, Example
53: designer nirA-promoter-controlled NAD(P)H-dependent Alcohol-
Dehydrogenase DNA construct (1510 bp)

```

<400> SEQUENCE: 53

```

agaaaatctg gcaccacacc cttctgacg aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcgaate gagaactgcc taatctgccg agtatatggt agctacctct 120
gtgccagaaa cccaaaaggg tgttatttct tatgagaatg gtggtaaat ggaatacaag 180
gacattccag ttccaagacc aaagccaaat gaaatcttga tcaacgtcaa gtactccggt 240
gtgtgtcata cggatttgca cgcattggaag ggtgactggc cattgccaac caagttgcca 300
ttggtcggtg gtcacgaagg tgctgggtgc gttgttgcct tgggtgaaaa cgtcaagggc 360
tggaacattg gtgactttgc gggtatcaaa tgggtgaacg gttcttgcct gtcctgtgaa 420
tactgtgaat tgtccaatga atccaactgt ccagatgctg acttgcctgg ttacaccacc 480
gatggttctt tccaacaata ccgtaccgca gatgctgttc aagctgccag aattccaaag 540
ggtaccgatt tggctgaagt tgctccaacc ctatgtgccc gtgttactgt ttacaaggct 600
ttgaaaagtg ctaacttgaa ggctgggtgac tgggttgcca tctctggtgc tgcctgtggt 660
ctaggttctc tagctgtcca atacgccaaag gccatgggtt acagagtcgt tggtatcgac 720
ggtgggtaag aaaagggtaa gttggtcaag caattgggtg gtgaagcctt tgttgatttc 780
accaaaacca aggacatggt tgctgaaatc caagaaatca ccaacggtgg tccacacggt 840
gtcattaacg tctctgttct tgaagctgcc atgaacgctt ccaactcaatt cgtcagacca 900
actggtagtg tcgtattggt cggtttgcca gctgggtgccc tcatcaagtc cgaagtcttc 960
tcccacgtcg ttaagtctat taacatcaag ggttcttacg tcggtaacag agctgacacc 1020
agagaagcta tcaacttctt cgctaacggt cacgtccact ctccaatcaa ggttgttgggt 1080
ttgtccgaac taccaaagggt ttacgaattg atggaacaag gtaagatctt gggtagatac 1140
gttgttgaca cctccaacta gggctgctgc cccggctgct gctaatagtg atcccggccg 1200
ctactaaagc ctgatttgc tttgatagctg ctctgcctt tgggcagggg cttttttctg 1260
tctgccattc ttgaggatgg cggactcttt cctttttgct ctacgcccat gaatgcgatc 1320

```


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```
gcagtcctccc ctgtccagca cgttgaggatg attggtgggtg gccagttagc ttggatgctg 1380
gcaccagcag cgcaacagtt ggggatgctg ctgcacgttc aaacacccaa tgatcacgac 1440
ccagcagtag cgatcgcgga tcaaaccgta ttagcagcag ttgctgacgc ggttctctct 1500
tctgccgtta 1510
```

```
<210> SEQ ID NO 54
<211> LENGTH: 56
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 54, Example
54: designer selected Hyd1 transit peptide (amino acids sequence)
```

```
<400> SEQUENCE: 54
```

```
Met Ser Ala Leu Val Leu Lys Pro Cys Ala Ala Val Ser Ile Arg Gly
1           5           10          15
Ser Ser Cys Arg Ala Arg Gln Val Ala Pro Arg Ala Pro Leu Ala Ala
20          25          30
Ser Thr Val Arg Val Ala Leu Ala Thr Leu Glu Ala Pro Ala Arg Arg
35          40          45
Leu Gly Asn Val Ala Cys Ala Ala
50          55
```

```
<210> SEQ ID NO 55
<211> LENGTH: 45
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 55, Example
55: designer selected RbcS2 transit peptides (amino acids
sequence)
```

```
<400> SEQUENCE: 55
```

```
Met Ala Ala Val Ile Ala Lys Ser Ser Val Ser Ala Ala Val Ala Arg
1           5           10          15
Pro Ala Arg Ser Ser Val Arg Pro Met Ala Ala Leu Lys Pro Ala Val
20          25          30
Lys Ala Ala Pro Val Ala Ala Pro Ala Gln Ala Asn Gln
35          40          45
```

```
<210> SEQ ID NO 56
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 56, Example
56: designer selected ferredoxin transit peptide (amino acids
sequence)
```

```
<400> SEQUENCE: 56
```

```
Met Ala Met Ala Met Arg Ser
1           5
```

```
<210> SEQ ID NO 57
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 57, Example
57: designer selected CFOCF1 subunit-a transit peptide (amino
acids sequence)
```

-continued

<400> SEQUENCE: 57

Met Leu Ala Ala Lys Ser Ile Ala Gly Pro Arg Ala Phe Lys Ala Ser
 1 5 10 15

Ala Val Arg Ala Ala Pro Lys Ala Gly Arg Arg Thr Val Val Val Met
 20 25 30

Ala

<210> SEQ ID NO 58

<211> LENGTH: 1417

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 58, Exmample
 58: designer nirA-promoter-controlled NADPH-dependent
 Glyceraldehyde-3-Phosphate Dehydrogenase DNA construct (1417 bp)

<400> SEQUENCE: 58

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg      60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat     120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat     180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgacceca ttgaagacaa     240
gggcaacaac catgacttgc acttacagtt tcttttgatg tcaaaagtgc tccaatttgc     300
tcagcaacat ctacaactct atttgaataa cccattcat tatcatacca agcaataact     360
tttactttat tccttgacat gaccattggt gattttgcat caataatagc tgaatttggg     420
ttagtattaa aatcaacaga cactagtggt tgatgttcga cttctatgat accttctaaa     480
cctgcatttt caaaagcttg gtttacttct tctgcagtta cttcttttcc taaatcaaca     540
actaaatcaa cgagcgatgc attctttggt ggtacacgta atgccatgcc gtgtaattta     600
ccttctaatt ctggtaatac ttcttttaaa gctttcgcgc caccagtaga agtaggaata     660
atgctttcat tacatgaacg tgcacgtcct aaatctttat gtggattatc aatatttttt     720
tggtcatttg taatagcgtg aacagtagtc attaaacat taactattcc aaactgatta     780
tttaaaactt ttgcaactgg accaatgcaa ttagtagtac atgaagcatt actaaaaatg     840
tcaaatgctt ctatatctaa ttggttatca tttacgcctt taactaacat ttgaacatgt     900
ccaccttttg aaggaccagt taacaatact tttttggcac ctgctttaat atgtgcatg     960
gctttatcac catgattaaa tttaccagtt gcattctatag caatctgat atctaattct    1020
ttccatggca agttttcagg attgcatca gcaaccaatt taattttatg atcaccaact    1080
tgcaatccat tttcaatcgg ttcaactttt agattatatt ttccatgtgt tgtatcgtaa    1140
ttgattaaat gtgcaattgt ttcgggtgga taactagcat ttatcgctac tacattttaa    1200
tttttatttt gtaatgcaat acgtaatacc attcttccaa ttctacccat accattaatt    1260
gcaatattcg ttgacattaa ggctgagatc ttcttcagtg cattgtagtt gaatgaaggg    1320
ttagggggga aatgcccccc tattttttgt ctagccatcc tgccacgttt gacagggtag    1380
caatttcgac acgatagggt tctctcttct gccgtta                                1417

```

<210> SEQ ID NO 59

<211> LENGTH: 1387

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 59, Exmaple
59: designer nirA-promoter-controlled NAD-dependent
Glyceraldehyde-3-Phosphate Dehydrogenase DNA construct (1387 bp)

<400> SEQUENCE: 59

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg      60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat      120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat      180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa      240
gggcaacaac catgcttaga gatgtgtgcg atcagatcca gaactttgtt ggagtagcca      300
gtttcgttgt cataccaaga aaccagtttc acgaagttgt cgttcagaga gataccggcg      360
tcggcatcaa acacggagggt gcatacttcg cgttgaagt cggtagaaac gacggcttcg      420
tcggtgtagc ccagaacgcc ttctatttcg ccttcagaag cggccttcac cactcacag      480
atctgctgat aggtcgcggg cttggccagg cgagcagtea ggtcaacaac ggagacgttc      540
ggggtcggaa cgcggaagc cataccggtc agtttgccgt tcagctccgg gatgaccttg      600
ccaacggcct tggctgcacc ggtagaggac gggatgatgt tctggctagc gccgcggccg      660
ccgcgccaat ctttcatgga cgggcgctcg acagttttct gggtcgcggg ggtagcgtga      720
acggtggtca tcagcgttc aacgatgccg aagttgtcgt tcaggacttt agccagcgga      780
gccaggcagt tgggtgtgca ggatgcgttg gaaacgatct cctggccagc gtagttcttg      840
tggtttacgc ccataacgaa catcgggta gcatctttag acgggcccagt catgacgact      900
ttcttggcac cggcggcgat gtgcttacgc gcggttctgt cggtcaggaa cagaccggtc      960
gcttcggcaa caacgtcaac gccgatttcg ttccacttca ggtagccgg atctctttca     1020
gcggtaacac ggattttttt accgttaacg atcaggtggc catctttcac ttaacagtg     1080
ccgttgaaac gaccgtgagt agagtcgtac ttcagcatgt atgccatgta gttggcatcc     1140
agcagatcgt tgatgccaac gatttogatg tcagaacgtt cctgagcagc acggaaaaaca     1200
atagggccga tacggccaaa accgttgata cctactttga tagtcattaa ggctgagatc     1260
ttcttcagtg cattgtagtt gaatgaaggg ttagggggga aatgcccccc tattttttgt     1320
ctagccatcc tgccacgttt gacagggtag caatttcgac acgatagggt tctctcttct     1380
gccgtta                                           1387

```

<210> SEQ ID NO 60

<211> LENGTH: 1627

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 60, Exmaple
60: designer nirA-promoter-controlled phosphoglycerate mutase DNA
construct (1627 bp)

<400> SEQUENCE: 60

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg      60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat      120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat      180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa      240
gggcaacaac catgggcccc gaacttttgc agcttgcccg cgtgcgccag caggagcggc      300
agcacgtggg tgccgtgcag gtcgcogagc ggcccccctg cgcctcgtc ctcggtgaag      360

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```

cgggggggccg cgtcggcgcg caggttgggg ccgtagagca ggaagggcac cgggtgccag 420
gagtgcgcct tcatcacgct gggcgtggag tggtcgccgg tgatggcgat cacctcgggc 480
tccagcgcca gcagctcggg cagcagggcg tcgaaggcct cgatcttggt caccttcggc 540
tcgaagtgcg cgtcctcgcc ggtggagtcg gtcttcttga agtgcaggta gaagtagtcg 600
aaggcttccc agtgggcctt cagggcccgcg agcttgccct cgggggcgctc ggggtcctcg 660
cccacctga cctccagcgc ggtcatcccc accagcgagg ccaccccgcg gtacatcggg 720
tagctggcga ccgcccgggg ggtgagcccg gtgatctcgc ccagggtggg ccagaccggg 780
cgcttgagga cgcgcgaag cagcaccccc ttgatcctgg gctcgtccgc gagcgcctgc 840
cgcgccagcg cgtgaaactt gttgagcagc tcggccgtct tggcgtggg ctctcgtctt 900
tcgtcgtgcg gacggggcgc gagcggcgcc accccggtct tctgggggtc ggtgctcgtg 960
accggtcgc ccagccccac gcccgcgagc accagcacga agcgggtgctc cgactcggty 1020
tggagctcga tcctgacgcc gtcgatctcg cggatgcgct ccctcagctt ggcgagacc 1080
cgccggtttt cctcgttggg gggggcgccg gcgcccgggt cggcgatggt gccgtcgggg 1140
ttcaaggtgg cgaagtggc gcgcaccgcg acgtcgtcgg gccccagctc gatgcccagc 1200
cccagcgccg agaggacgcc gcgcccgacc tcgtagcggg aggggtcgtg gccgaagagg 1260
ctcaggtggc cggggccgga gccgggcgcg aagccggggg ccaccagggt gactcgcgcc 1320
aggttggcct ttccgcccag gccgtcgagg ttgggggtgc gggccgccc cagctccgtg 1380
ggcccggccc gctcgggggg cagcccggcc accccgtcga gcacgacgaa gaggatcttg 1440
ctgggcgtgg tcgggctcag ttccttgagg tgggggagga ggtccattaa ggctgagatc 1500
ttcttcagtg cattgtagtt gaatgaaggg ttagggggga aatgcccccc tattttttgt 1560
ctagccatcc tgccacgttt gacagggtag caatttcgac acgatagggt tctctcttct 1620
gccgtta 1627

```

<210> SEQ ID NO 61

<211> LENGTH: 1678

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 61, Exmample
61: designer nirA-promoter-controlled Enolase DNA construct
(1678 bp)

<400> SEQUENCE: 61

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtttttg taaattatag aaaacccccca acccaccgata cagcccgata 300
tcgcttaatt cttcttctat ccgcaacagc tggttgtatt tcgctaccgg gtcagtctcg 360
gccggagccc cggctcttat ctgcctcagc ttggtcgcta ctaccaggtc agegatgaag 420
gtgtcctccg tctccccgga acgggtgggat actacacagg tgtaaccggc tcttttagcc 480
atttcaatcg tatccaaggt ctcagtgacc gtgcctatct gattcagttt gataagtatg 540
ctgttggcca cacccttttt tattcctgtg gtaaaacgct ggggattggg tacgaaaata 600

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tcacatccga	cgatctgaat	cttcttgccc	agcttacggg	tgagcttttt	ccacccttcc	660
cagctcttct	cctgcagccc	gtcttocagg	gatatgatag	gatacttacg	cacaaggcca	720
tcgtaaaact	cgaccacctc	atcgcggtc	agctcttggc	cggtgctggc	aaaaacgtat	780
ttcccgctct	tgaaaatctc	attggcagca	acatccagtc	ccaggtaaat	atccttcccc	840
ggcttgtagc	ccgccgtttt	aatagcctcc	aaaattactt	caatcgcggc	ttcattcgaa	900
ggcaagttgg	gagcaaatcc	tccttcatcc	cogatggaag	tagaaaaacc	ttttttactc	960
aataccctct	tcagggtatg	gtagacctcc	acccccatgc	gcagggcctc	ggcaaaagaa	1020
gtagctccta	ccggttaggat	caagaattct	tgaatgtcca	cattattatc	cgcgtgcttg	1080
ccgcgcttca	agatgttcat	ttgcgggatc	ggcagttctt	tggcgtttac	gccgccccagg	1140
tactggtaaa	gcgcatgga	taggtaggaa	gcagcagccc	gcgccacagc	catcgacacg	1200
cctaagatag	cgtagctccc	cagcttgccc	ttggtgctag	tcccacgag	gtcaatcctc	1260
aaccggtcaa	tccccacctg	gtcagcgcga	tccatcccca	caacctccgg	ggcagtgacc	1320
gtgttgacgt	tatccaccgc	attcagcagc	cctttgccgc	cgaaacgctc	tgcgtcccca	1380
tcccgagtt	ccaccgcctc	aaaagcacca	gtggacgcgc	cggaaaggaac	cgcagcccgt	1440
cccatggtcc	catcttctaa	aaggacctcg	acctccaccg	tcgggtttcc	ccgcgaatcc	1500
agtatttctc	tggcgtaaac	ctcggtgata	atactcacta	aggtgagat	cttcttcagt	1560
gcattgtagt	tgaatgaagg	gttagggggg	aaatgcccc	ctattttttg	tctagccatc	1620
ctgccacggt	tgacagggta	gcaatttcga	cacgataggg	ttctctcttc	tgccgtta	1678

<210> SEQ ID NO 62

<211> LENGTH: 2137

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 62, Exmample
62: designer nirA-promoter-controlled Pyruvate Kinase DNA
construct (2137 bp)

<400> SEQUENCE: 62

agaaaatctg	gcaccacacc	tgacccccat	cgagagactc	cgaacgtggc	aaatggaggg	60
accagagttg	ttcagttcac	aggtagataa	tgtcgcgggt	cttgatagtt	agcaataaat	120
acagtttcag	aatatctgta	atacaaaaac	tgtatcgaga	caagaaaaaa	gtagcaaaat	180
ttacaaatgt	tcatgattca	tctggctaaa	ttggatgttc	aactgaccca	ttgaagacaa	240
gggcaacaac	catgccgcac	ttgggcttgg	ccgcggtaga	tttgtcccct	agccgtatcc	300
atggttatgg	tttctccgct	ttggatgaca	cgggttgctc	cggcggctcc	aacgatgaca	360
ggaatatcca	agttaatacc	tactatggct	gcatgagaag	tcaggcctcc	ctgttccgtg	420
ataatcccc	cggccttttc	caacaacggc	atatagtctc	tgtctgttcc	gactgccacc	480
actatatctc	ccggagcaaa	cgagcccggc	ttgcccggat	ccagtatcac	ccgcgcctta	540
ctggtaacgg	cccgcttacc	tatacccggt	cccttaacca	gcaccttgcc	tactacttga	600
actctcagta	gattgtagt	cccagggatt	ccaggagtac	ctgcggttat	gactaccagg	660
tcaccggggg	agattagccc	tcgcctgact	gcggcatcca	gggctgtttc	tatcatctgg	720
tcagttccgt	gggtttccgg	gaccgtgagc	gcgtataccc	cccataccaa	agttagcttg	780
cgtaccactt	cgggtgaagg	cgtagtagcc	acgatcggag	cccggggacg	gtacttggcc	840
accattctcg	ccgatggccc	cgattttgta	gcagtaacaa	tggccgaggc	cccaggtc	900

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gtagcggtag cacaggtggc ataactgatg gcacgggtca cggtagcct gccttcgaat 960
cgtcttgccg ccagcatggt ctcatagggc aaagccatct ccgtccgccc ggctattcgc 1020
gccatggttt ccaccgcaac taccggatat ttaccggctg cggctctctg cgacagcata 1080
atggcgtctg cccctcaaa tatggcatta gccacgtctg acgcttcggc ccgggtgggg 1140
cgcggcacgt ttaccatcga ttcgagcctc tgggtggcaa ttatcaccgg cttaccctga 1200
gcccggcatt tctcgattat aaccttttgc accagaggta cttcttcggg aggtatttca 1260
acccccaggc ctccccgggc gaccatgacg ccacagcaa cctttattat gtcacccagg 1320
ttgtccagcc cctcctggct ctcaactcta gcgattatat cgatgtcggc tcccttttcc 1380
tctaatatgc gtctgatatc caaaacatca tcggccgtcc ttacgaacga ggcagcaatg 1440
aaatccatat tctgtggat gccgaagtta atgtcttcga tgtctttctg gctcaaaaaa 1500
ggcaggttgg ttctcacacc cggcagggtt atgcctttcc tttcaccgag tactcctccc 1560
gcaaccacct ggcagacgat gtcgggtctg ttcgecteta aaacagacag ctggatgacg 1620
ccgtcagcga tcaatatgca gtcacccgct tttactctg aagtaactc atgtaactt 1680
atctggacct cgtctcgtt accttcaacc ggacgggttg tgagaacgaa cttttgacca 1740
ggacgcagct ctatcttacc ttctttcaag ggcccggctc tgatctccgg ccttttggtg 1800
tcaagcatca acctacttc cgcattcagt tcccgcgcca cctcacgcac catgcccgatg 1860
cggcgctcat gctcatcata agtgccgtg gaaaaattca aacgggccac gttcatgccc 1920
ttggtgatca aagctcttag ccgctcataa tcacgggtgg aaggctccag ggtacagatg 1980
atthtggctc tccgcaataa ggctgagatc ttcttcagtg cattgtagtt gaatgaaggg 2040
ttagggggga aatgcccccc tatttttgt ctagccatcc tgccacgttt gacagggtag 2100
caatttcgac acgatagggt tctctcttct gccgta 2137

```

<210> SEQ ID NO 63

<211> LENGTH: 2163

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 63, Example
63: designer nirA-promoter-controlled citramalate synthase DNA
construct (2163 bp)

```

<400> SEQUENCE: 63

```

agaaaatctg gcaccacacc tgatctgcaa gagacgctca cgcgatacct ctgggcgtgc 60
aatcaccgca ttcccccaac cgctttgggc cacatgacct ccacgagag actccgaacg 120
tggcaaatgg agggaccaga gttgttcagt tcacaggtag ataatgtcgc gggctctgat 180
agttagcaat aaatacagtt tcagaatcgc tgtaatacaa aaactgtatc gagacaagaa 240
aaaagttagc aaatttcaaa atgttcatga ttcactctggc taaattggat gttcaactga 300
cccattgaag acaagggcaa caacctgga gcaggttttt atctacgaca ccaccttgag 360
ggatggctcg caggcagaag gtataaactt ttccgtagag gataagatgc gcatacttca 420
aaaaactggc gaatttggag tgcattacat agagtgcgga tggcccgggtg cgaacccaaa 480
agacactatt ctctttgaaa ggctgagaaa gataaaaact caaaatgccca aaatagtagc 540
ctttggtgca acaagaaaag ctggaaaagaa ggcgcacgaa gataagcagg tggaaaacct 600
ttgaaatcg ggtgccaagg tgataaccgt atttggcaag agctgggact tcatgtaac 660

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gcatgccata gggaccacct tagaggaaaa cctggacatg gtttacgaga cggtaagcta 720
tcttaaaaag catgtggagg aggttatcct tgacgcagag cacttctttg acggatacag 780
gcacaacgaa agctatgctt ttaaggtatt ggaggcagct tttcaggcag gtgoggactg 840
gatagtccct tgcgatacca acggtggcac ccttcccaat gaggtttatg agataaccaa 900
aaaggttgta caaaagtctc cacaggcacg cgtaggcata cacgctcaca acgattcaga 960
tactgctgtg gctaactctc ttatggcggt gcttcagagt gcaaggcagg ttcacggcac 1020
tataaacggc ttgggggaaa gaacgggcaa tgctaactctg tgtccataa tacctaacct 1080
tcagctcaag ctgggcttta gtgtagtgcc ttcccaaac ctcaaaaagc tcaccgagct 1140
tgctcacttt gtctccgaaa tctccaacac gccactgccc aaaacatgc cttatgtagg 1200
ggagagtgct tttaccaca aagcaggcgt acacgcctct gcagttatga aaaggtcaga 1260
aacatacгаа cacatagacc cttctttggt aggaacaga aggaaggtga cagtgtctga 1320
cctttctgga aggagtaata tactttacaa gctcaggaa atggggcttg aggtggatga 1380
taagtccctt gagcttatca aactccttga aaagataaag gaacttgaga aggaaggcta 1440
ccactttgaa gcagctgaag cttcttttga gcttctttgc aagaggcatt ttgggcttgt 1500
taaaaactat tttgaccttg atgcttacag ggtgctaata gccagaagga gtacagacct 1560
atctcctggt tcggaagcca ccgtaagact ctatgtggaa gacataaagg agcatacagc 1620
agctcttggt aacggaccag tgagcgccct tgacagagcc ctcagaaaag ccttggaga 1680
gttttatcca agccttaaag atgttcagct catagactac aaggtgagaa tagttaacga 1740
atcggagggt acatctgcca aagtgagggt gcttatagaa tctaccgatg gtagaagaaa 1800
gtggggaacg gtgggagttt cggaaaacat aatagaagcc tcttggatag ccttaactga 1860
tagcctcgta tataaactct taaaagacga agaagagggt ataatgtgat aaggctgaga 1920
tcttctcag tgcattgtag ttgaatgaag ggtaggggg gaaatgcccc cctatTTTT 1980
gtctagccat cctgccacgt ttgacagggt agcaatttcg acacgatagc gtgctgtact 2040
gttttttctc cgtcagggtt gggttttgtc atcgacaccc aaggattgga gtcggtgctc 2100
aataatcgcc agttgctggt gggcagccgc caattgcgcc tgaggttctc tcttctgccg 2160
tta 2163

```

<210> SEQ ID NO 64

<211> LENGTH: 2878

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 64, Example
64: designer nirA-promoter-controlled 3-Isopropylmalate/(R)-2-
Methylmalate Dehydratase DNA construct (2878 bp)

```

<400> SEQUENCE: 64

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcggtt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgatcgaga caagaaaaaa gtacgaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtaccat gtctgctggt gctatctcgc ccttgattgc tgatgctgta 300
acagtagccg ctgaagcaag atatacaaaa gaatctttat gtctgcacg tcccttgaag 360
ttcgtgtac ctgtactgat aagagtctca cctcaccga taacaccctg acagcttccc 420

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cagcatacag agcagttagg attcataaca attgcacctg cgtccatgaa tatatcaagg	480
agtccctcct tcatagcctg aagatatacc gaacggcttg caggaactac aaggaatcct	540
accttaggag caaccttttt ccctttgatg atcgetgctg caactcttaa atcctcgatt	600
cgtcattgt tacatgaacc aagaaatgct tcatcaatct ttacaccaag tgattcctta	660
gccggaacta cattgtcaac aaaatgtggc tttgcaaca ttggctgtat tgttgaaagg	720
tcaatatcat aaacctgctc aaatactgca tcatcatctg atgtaaagca tgcctttggc	780
tctctgcat gctccttaag ataatccatt gcaacatcat caacttccat gagtgcagtc	840
ttagcacctg cctctacaca aaggttacag attgatattc tgtctgcat tgaaggctg	900
tgtaagcctt ctctgcaaaa ttccattgct ttatagttag caccgttagc gccaatcttt	960
ccaataatag agagtattaa atctcttgca tatactccat cgttaagctt tcccttaagg	1020
ttgaatctta atgttcccg aaccattacc catgatgttc ctgtaacct tgcatacaaaa	1080
taatctgtac aaccaacacc tgtaccaaat gcacctaacg caccatagc acaagtatgg	1140
ctgtctgctc caaataaag ctcccccgc actacatgat tttccatcat aacctgatga	1200
cacacaccct cgcctcgta gaacttaata tcattagcct tagcaaagtc tctcatcttc	1260
ttctgtgagg ctgctgtctt aggactgtct gatggaatat tgtggtctac aatccataca	1320
agcttatcct tgtcagcaat atgaggattc ttttaactct catacatacc aatagtaaga	1380
tgtgtgttc catcattact cataagtctg tcaagagtaa cagttgcaat atcaccagcc	1440
ttaacctgtg aaagacctgc tgccttgcg ataactctct ctgcaatagt catgccatgc	1500
tttgctcat ctgcaggtae ggctgtactc tcagactctt ctttctcacc atcaagtat	1560
gcaataagac caccctgatt aagaatagcc tgcactctgg ctggaagctt agtacatgta	1620
taagtcttc cattaacagt tataattcca tcttctaag aaagctcaca ttcaccccc	1680
gcattaactt cgtcatggag ttctttacat acaataacag gaagtcctat attaatagca	1740
ttacgataga atattcttgc aatgatttg gcaatcactg ccttgacacc taatgcctta	1800
agtacgcttg gtgcctgctc tcttgatgaa ccacatccaa agttgtcatc tgcacaacg	1860
aatctcccc gctttacggc agaagcaaaa tcagagtcta atgattcaaa tgtatgactc	1920
ttcatctcat caattgtcgg aaacaaaaga tactgctgat caataatctg atctgtatca	1980
acatctttat caaacttaaa tatcttacc attgaccccc atcgagagac tccgaacgtg	2040
gcaaatggag ggaccagagt tgttcagttc acaggtagat aatgtcggg gtcttgatag	2100
ttagcaataa atacagtttc agaatatctg taatacaaaa actgtatcga gacaagaaaa	2160
aagttagcaaa atttacaat gtctcatgatt catctggcta aattggatgt tcaactgacc	2220
cattgaagac aagggcaaca accatggtca agaccgttaa gctttctcat tgccttaaca	2280
agtccgctg cattaagtat atccacaaga ttatcaggaa gtgaagcaat aggatagct	2340
ttccattgt gtgtaatctt tgcatttact tcaacatcaa tagtatgcc tccgtaact	2400
tcgtcgtgaa ggtctgcatt ctctataagg agaagtcctg tattaataga atttctgaag	2460
aatattcttg catatgattt ggcaataaca catttaatac ctaatgcctt aataacctca	2520
ggtgcctgct ctcttgatga accacaacca aagttcttct ctgcaacaat gatgtcgcct	2580
ggcttaatct gacctgcaag ttctggctt aatggcgaaa atgcatatgg tttcatatct	2640
tctactgtct ttaatgcaag gtactctgta gggataatga tatctgtatc aatgcatca	2700

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ccaagtacc atactttacc gctaaatttc tegtccatta aggctgagat cttcttcagt 2760
gcattgtagt tgaatgaagg gttagggggg aaatgcccc ctattttttg tctagccatc 2820
ctgcacggtt tgacagggta gcaatttcga cacgataggg ttctctcttc tgcctgta 2878

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<210> SEQ ID NO 65

<211> LENGTH: 2380

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 65, Example
65: designer nirA-promoter-controlled 3-isopropylmalate
dehydratase DNA construct (2380 bp)

```

<400> SEQUENCE: 65

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacia 240
gggcaacaac catgaatgaa tcttcttggg tctgtgatgt atccggttac cgcgggtggt 300
gccgcgcttg caggagaagc aaggtatata tccgcattgg gattccccat ccttcccttg 360
aagtttctgt tctgcgtgga aagtaccctc tctccgtctc caagaacacc catgtggatt 420
ccaacacatg ggccgcaacc tgggtgata actgcccctc cgagttcaac aaatttcttg 480
attattccct tttcaagggc gtccatgtag accttccctg aagcggggcc gacgatcagc 540
ctcacatccg ggtgctttcc gtgtttctca agaattttca aagcgatctc aagatcctga 600
agtcttccgt tcgtagaggt tccatgaac acctgateta tctttatctt ttccttttca 660
acctcgctca cctttctcac gttgtccaca tagtgaggca aagagacgag tggttcagat 720
gtgtcggcat ctatctctat ctctgtctcg taaaccgctg ctggatctgc tttcaactct 780
ctgaagtcct cctctcttcc catcttttcc aggaactctc tggctctctc atcagaaggc 840
atgagacctg ctttctctcc cacttccacc gccatgttgg aaatggtgag tctgtccctc 900
acattcatat ttctgataca gcttccatgg aactccaacg ctttgaagt tgcgcccctg 960
cttccagaaa ttctcggcat ctcgagaatg atgtctttcg cgtaaactcc atcctgtaac 1020
ttcccgttca ccacaacctt gatcgtctca ggtactttga accagttctg tccaagcccg 1080
aagatgatcg caacatctgt ggacccccatt cccgttccga aagcaccgag cccaccggca 1140
gtgcagggtg gcgaatccgc acctgctacc agatcgccgg gtttcacgta tttttccgag 1200
aggatctggt gggatatccc gtctcccga tcgaaaacct tgactcccat ctcttttcca 1260
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tcgatgaaga ggaaggcctt cgggaacctc acttctttga agccgagttc tctgaattcg 1380
tttatcatca gggggcctgt tccatcctgg gccatggcta tatccactct cgcgagtacg 1440
atcttccggc ctttccagtc tcttccagta tgttcagaaa agatcttttc tgcgagtgtc 1500
ttaccattg acccccatcg agagactccg aacgtggcaa atggaggggac cagagttggt 1560
cagttcacag gtagataatg tgcggggtct tgatagttag caataaatac agtttcagaa 1620
tatctgtaat acaaaaactg tatcgagaca agaaaaagt agcaaaatct acaaatgttc 1680
atgattcatc tgggtaaat ggatgttcaa ctgaccatt gaagacaagg gcaacaacca 1740
tgaactcttg ggaaggaacc gtgcttttcc agatagttca ctataccgtc tcttttcagt 1800

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atctcgagga gaaacttcgg aatcggagtg aatctgtatt ctttctctgt tgtgaggttc 1860
ttcaaaacac cgttttcgag atctatctca agttcgtctc cctggttgat ctctcgact 1920
tccttgagct ctatgactgg aagtccaca ttgatggcgt ttcggtagaa gatccttgcg 1980
aaagacttcg ccacgataca ggaaacacca gogatcttta tgatacgcgc agcgtgctct 2040
ctggaagaac caagtcgaa gttcttgcca gccacgatga tgtcaccttt ctgcaccttc 2100
ttcgcgaaat cctccatggc atcttccaag acgtgttttg cgaactcttc aagattgttc 2160
ctcagatgaa aataccttcc aggtgctata tggtcagtcg atatattgtc accgaatttc 2220
cagactcttc cccttatcat taaggctgag atcttcttca gtgcattgta gttgaatgaa 2280
gggtagggg ggaaatgcc ccctatctt tgtctagcca tctgcccacg tttgacaggg 2340
tagcaatttc gacacgatag ggttctctct tctgcccgtta 2380

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<210> SEQ ID NO 66

<211> LENGTH: 1456

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 66, Example
66: designer nirA-promoter-controlled 3-Isopropylmalate
Dehydrogenase DNA construct (1456 bp)

```

<400> SEQUENCE: 66

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgccatat ttcttogagt tttttacata tgagatctcc catctgagag 300
gtcgaaaccg ccttttctgg atcctctgcg atgtctctgg ttctgtatcc ctcttctatc 360
accagctcaa ccgctcttcc tatcttctct gcctcttcca ccattccaaa ggaatgctcg 420
agcatcatgg cgagagagag gatctgtgcg atcgggttgg cgatgttctt tccggctata 480
tcaggagcgg aaactctgce cggctcgtag aggttcttat caccgaaaga cgcggacggc 540
agaagaccaa gagaaccagg aagtgccgca ctctcatccg agagaatgtc tccaaacatg 600
ttcgttgatg ggatcacatc gaactgcat gggttcagga tgagctgcat ggcagcgttg 660
tccacataca tgtgcgtcag ctccacatca gggattctc tcgctacttc gttcacaact 720
ttctccaca gcatggaact gtagaggacg ttcgcttctg caacggaggt gaccttttt 780
cttctgtttt ttgcgatttc aaaggcagtt ctgcgatcc gttccacggg ttttctgctg 840
tagatcatgg tgtcgaatcc cttttcttca tccaatcccc tcggctggcc gtagtaaaact 900
ccgtaggaaa gttccctgac ggtcacaaga tcgaccccg atccaatcac ctttctttc 960
aaaggagaga catgcacaag cgatctgtag acctttatcg gtcttatggt tgcgtaaagg 1020
ttgagcatct tccttagggc aagaagcccc cctatttccg gcctcttctc cggaggaaga 1080
tcgtcccatt taggtctctc gacgcttcca aggaagatcg cgtcggttc cagacatctc 1140
ttttttgtct cttcaggaag ggggtccacg aatttgtcta tggcatcccc tccgatgtgt 1200
caaagactt tetcaaagg tttccccgtt ttcttttcca ccacctcgag cactttaaga 1260
gcttccctta caacctcggg acctatgccc tctccaggca aaaccgctat cttcattaag 1320

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gctgagatct tcttcagtgc attgtagttg aatgaagggt taggggggaa atgccccct 1380
atTTTTtTgc tagccatect gccacgtttg acagggtagc aatttcgaca cgatagggtt 1440
ctctcttctg ccgтта 1456

```

<210> SEQ ID NO 67

<211> LENGTH: 1933

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 67, Example
67: designer nirA-promoter-controlled 2-Isopropylmalate Synthase
DNA construct (1933 bp)

```

<400> SEQUENCE: 67

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacia 240
gggcaacaac catgctcagc acccccgttt tttctgagaa ggcccttagc gatgaggtac 300
ttgtttattg cgtttatgta agctatcgca gaggttcca ctatgtccgt ggagacaccc 360
cttccactgt agagttcacc gtttattctc aacgtgagtt tgacctctcc ctgtgcgttc 420
tttccagttc caaccgctg aattatgtac tctcagagct tcggttgaat accgagcgtc 480
ttgtctatag ccttgaagat agcatccact ggaccgttcc ctgcctctgc tgcctctttc 540
ttttcatctc caacctgaag cacgaccgcc gcggttgaa gcagcgtggt tccggtgtgt 600
acatggaagt gaacgagctt gtaaccgttg atgggctccc tcaaaacttc cgagacgatc 660
gagaaaagat catcgtcgta aacctcttc tttctgtcgg cgagtctgtg gaacttctcg 720
aacactttct ggaaggtctc ttcgtcagat ttgatgccgt agctctccag cttctttctg 780
agggcggtct ttcggagtg tcttccaagc acgagcgtct cggagacct gccgatatcg 840
gatggtttca tgatctcgta ggtctccctg tgtttcagca caccatctcg gtgtataccc 900
gactcgtgaa ggaacacggt ctctcccact atgggtttgt ttctggacgg gatgagcccc 960
gttatatgty tgaggagcct ggaagcgggg tatatgagct ctgtctttat acccgtctcg 1020
tagggaagtt tgtctttcct caccttgagg atcatcacga actcttccag ggcacagttt 1080
cctgccctct ctccgatacc gttcagagtc acttcgacct ggggtggctcc gttctgaacg 1140
gcagcgaggg agttcgccac agcaggtcca agatcgttgt gacagtgcac agaaagatcg 1200
acattctcta taaccggcac accctctctc aaggtcttta tgagttctcc aaactcatcg 1260
ggaagggcgt accccaccgt gtcgggaaca ttgatcgttg tggctccggc ttcgatcgcc 1320
gtcttgtagg cttctatcaa aaagggaacc tccgttctcg aagcgtcttc cgcgagaaac 1380
tccacaaggt cgaaaaactg ttttgcgtag ccgacgtatc ttctgatcct ctcgaggatt 1440
tctctttctc ccattctcag tttgtatttt ctgtgaatcg gagaggtcgc tatgaaaaacg 1500
tgtatcatac gtttgtcttt tggctgatcc ttgagagcct cgtacaccgc gtctatgtcc 1560
ttttcaacac accttgcgag tcccaccact atgggtttct gaacggcgtc cgaaactctt 1620
tttacagctt caaactgcac gggagatgaa acgggaaacc cagcctcgat gagatcgaca 1680
ccgagatcct ctaacatgag tgccatttct actttttcct caaccgacat cgaagcccct 1740
ggggattgct ctccatccct caacgttgta tcgaagatct taattctcct cattaaggct 1800

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gagatcttct tcagtgcatc gtagttgaat gaagggttag gggggaaatg cccccctatt 1860
ttttgtctag ccatcctgcc acgtttgaca gggtagcaat ttcgacacga tagggttctc 1920
tctttgccc tta 1933

<210> SEQ ID NO 68
<211> LENGTH: 2632
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 68, Example
68: designer nirA-promoter-controlled Isopropylmalate Isomerase
DNA construct (2632 bp)

<400> SEQUENCE: 68

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatattctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgacagac cggctcggct tcaagctggc gcacatcgac aaaatgtccg 300
taaatcgag ccgcccgggc catcacgggg ctgacgagat gcgtgcgcgc ccttttctc 360
tgccgccctt caaagtgtgc gttcgcagtt gacgcacagt gctcgccttc tgggatgatg 420
tccgggttca tgcccaaaac ggcgctgcag ccggagtcgc gccattcaaa accggcgtcg 480
atgaaaaatt gtgccaaacc ttccgcttcg gcttgtttt tcaactgtctg cgatccaggc 540
acgacgagcg cccttacacc aggagccact tttttccctt tcacgatgct cgcgcgcgcg 600
cgaaaatcgc tgaggcgtga gttggtgcac gaaccgatga agacgtgctg caccggaata 660
tccgtaatcg gcgtgcccgg cttgagcccc atgtactcaa gcgcccggcg caccgcgttt 720
tgctctgttt tgctttcaaa ttgctcggga tgcggcacga ccgatcgac ggaagtgtc 780
atcgcggggt tcgtccccc cgtcaccatc ggagcgcgac tgcacgcac gatttcaatc 840
gttttatcgt attttgcccc ctcatcgtct gctaaccgcc gccaccgttc caccgccttg 900
tcaaactcct cgctttttgg tgcataattg cggccgcgca aataggcgaa cgtcgtttca 960
tccgactga tgaggccgac tctcgcacca gcttcaatcg acatgttga aatcgtcatt 1020
cgctcttcca tcgacatgag cggatcgtct tcgcctgtaa attcgataat ataaccggtg 1080
ccgacgcaaa ccgatagcgc gccgataatg gccaaagatga cgtctttggc ggtcactcct 1140
ttgcccaggc ggcccgtgat gcagatttgc agcgttttcg gcttatgctg ccaaagcgtt 1200
tgtgtagcca atacatgctc gacttcgctc gtgccgatgc caaacgcaaa ggcgcaaac 1260
gccccgtgag tcgacgtatg gctgtcgcgc caaacgatcg tttccccggc ctgggtcaac 1320
ccgagctctg ggccgatgac gtgaacgatt ccttgcctct cgctgtgtag gtcggcgagc 1380
ggaatgccga actcgcggca gttgcgctca agcgcagcga tttggttgcg cgccacttcg 1440
tcggtaatca caaatcgggt aacggttggc acgttatggt ccacgtcgc aaaggtcaaa 1500
tccggccgac gcaccttcgc tcttttttgc cgcaaccctt caaacgcttg cggcgaggtc 1560
acttcatgca ctaagtgcaa atcgatgtac aataaatccg gtttgccctc ctacgggtg 1620
acgacgtggt tttcccaaat tttatcgatg atcgttttcg gcttcattga ccccatcga 1680
gagactccga acgtggcaaa tggagggacc agagttgttc agttcacagg tagataatgt 1740

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cgcggtctt gatagttage aataaataca gtttcagaat atctgtaata caaaaactgt 1800
atcgagacaa gaaaaaagta gcaaaattta caaatgttca tgattcatct ggctaaattg 1860
gatgttcaac tgaccattg aagacaaggg caacaacat gcgccgtgg acaatgccgt 1920
cgttcgtagg cggcgatag cgcttcgtac acaaacgtca aatcaatttc gtcccatcct 1980
tttagcaaca gctgttttcg atacgggtcg atgtcaaacg gacgcgaaaa tccttcatcg 2040
tcaaataccc gctgttttc aagcgaacc gtcagttcat agtctgcgcg ctgctttgg 2100
cgcagcaagt agcggacatc ctctttatcc agccggatcg gcaacagtcc attttttaag 2160
cagttgttgt aaaaaatc ggcaaacgat ggggcaatga ttacgcgga tccgtaatct 2220
tgcagcgcgc acggcgcgat ttcgcgcgat gagccgcaac cgaagttttc atcggcgact 2280
aaaaatcgtc ccccttcgtt ttcggggcgg ttgagctcaa actccgatt tggcgtgccg 2340
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ttcaaaaact gttttggaat gatttgatcg gtatcaatat tcgcccgatc gatgccggcg 2460
gtttttccgc gatggatcgt aaacggcttc attaaggctg agatcttctt cagtgcattg 2520
tagttgaatg aagggttagg ggggaaatgc cccctatctt tttgtctagc catcctgcca 2580
cgtttgacag ggtagcaatt tcgacacgat agggttctct cttctgccgt ta 2632

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<210> SEQ ID NO 69

<211> LENGTH: 2035

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 69, Example
69: designer nirA-promoter-controlled 2-Keto Acid Decarboxylase
DNA construct (2035 bp)

```

<400> SEQUENCE: 69

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtataca gtaggagatt acctgttaga ccgattacac gagttgggaa 300
ttgaagaaat ttttgagtt cctgggtgact ataacttaca atttttagat caaattatct 360
cacgcgaaga tatgaaatgg attggaaatg ctaatgaatt aaatgcttct tatatggctg 420
atggttatgc tcgtactaaa aaagctgccg cttttctcac cacatttgga gtcggcgaat 480
tgagtgcgat caatggactg gcaggaagt atgccgaaaa tttaccagta gtagaaattg 540
ttggttcacc aacttcaaaa gtacaaaatg acggaatatt tgtccatcat aactagcag 600
atggtgattt taaacacttt atgaagatgc atgaacctgt tacagcagcg cggactttac 660
tgacagcaga aaatgccaca tatgaaatg accgagtact ttctcaatta ctaaaagaaa 720
gaaaaccagt ctatattaac ttaccagtcg atgttgctgc agcaaaagca gagaagcctg 780
cattatcttt agaaaaagaa agctctacaa caaatacaac tgaacaagt attttgagta 840
agattgaaga aagtttgaaa aatgcccaaa aaccagtagt gattgcagga cacgaagtaa 900
ttagttttgg tttagaaaa acggtaactc agtttgtttc agaacaacaa ctaccgatta 960
cgacactaaa ttttgtaaaa agtgctgttg atgaatcttt gccctcattt ttaggaatat 1020
ataacgggaa actttcagaa atcagttcta aaaatcttgt ggagtccgca gactttatcc 1080

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taatgcttgg agtgaagctt acggactcct caacaggtgc attcacacat catttagatg 1140
aaaaataaat gatttcacta aacatagatg aaggaataat tttcaataaa gtggtagaag 1200
atthttgattt tagagcagtg gtttcttctt tatcagaatt aaaaggaata gaatatgaag 1260
gacaatatat tgataagcaa tatgaagaat ttattccatc aagtgtctcc ttatcacaag 1320
accgtctatg gcaggcagtt gaaagtttga ctcaaagcaa tgaacaatc gttgctgaac 1380
aaggaaccte atthtttggg gcttcaacaa ttttcttaa atcaaatagt cgttttattg 1440
gacaaccttt atggggttct attggatata ctttccagc ggctttagga agccaaattg 1500
cggataaaga gagcagacac cttttattta ttggtgatgg ttcacttcaa cttaccgtac 1560
aagaattagg actatcaatc agagaaaaac tcaatccaat ttgttttacc ataaataatg 1620
atggttatac agttgaaaga gaaatccacg gacctactca aagtataac gacattccaa 1680
tgtggaatta ctgaaatta ccagaacatc ttggagcaac agaagatcgt gtagtatcaa 1740
aaattgttag aacagagaat gaatttggc ctgtcatgaa agaagcccaa gcagatgtca 1800
atagaatgta ttggatagaa ctagtthttg aaaaagaaga tgcgccaaaa ttactgaaaa 1860
aaatgggtaa attatttgcg gagcaaaata aatagtaagg ctgagatcct cttcagtgca 1920
ttgtagtga atgaagggtt aggggggaaa tgcccccta tttttgtct agccatcctg 1980
ccacgttga cagggtgaca atttcgacac gatagggttc tctctctgc cgta 2035

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<210> SEQ ID NO 70

<211> LENGTH: 1426

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 70, Example
70: designer nirA-promoter-controlled NAD-dependent Alcohol
Dehydrogenase DNA construct (1426 bp)

```

<400> SEQUENCE: 70

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgggggat caggactgcc ctgcctagaa cttcaccttt ctctagcctc 300
tcaagcacat cgtttatttc atctagcttg tggatatcaa cctctacctt gaccttacc 360
tgaagggcta acgtgactag ctcatggagc tctacatagt ttctactag gcttccttca 420
aaggatacct cagaggatat caccctgatc gtggggaatc taagctcacc cccatagcct 480
acgatgataa gcctccccat cctccctagc aggtatggtg tataatcgac ttagcctga 540
gagcctacga agtccattgc aacgttaact cctctcccc tggttaagctc catgacctgc 600
tttacagggc ctctctagc gtcaaccacg tgatccgctc caagcctctc ggccagcttt 660
agtttttctt ccttaacgct cagtgtatc accgtcgcgg gtgtcataac ttaagtagc 720
tgaactgcaa tatgacctaa tctctccacg cccactatag cgacgtatgc gccgggatag 780
agggttcggg cggccttctt aacagcccta taagccgcta tcccagcgtc cgctagaggg 840
gccatttcaa caagtttctc cctgctaata tcttaggca gctttatcac agacctgtgc 900
gaggctctca tgaactctgc aaatccacca tcgatattaa gtctgggaa ctctaggttc 960

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tcgcagtgca tatkctcacc agctctacag gctagacagg ttccatctgt gaccgcccgg 1020
tgaagaatta cggggteccc cttctctaag ccttccactc cttcggcaac ttcttcaata 1080
taccgacgt tctcatggcc taaagtgtag ggtagcttag gctgcaatag ctcatgccac 1140
attccctgga caaggtggag gtccgtatgg catacgccag cgccctgcaat ccttacaata 1200
acgtcaaate taccttctag cctcggatag tcgacatcct ctatcctcaa cggcttgta 1260
tactcgtgga gcctggcagc tttcaataag gctgagatct tcttcagtgc attgtagttg 1320
aatgaagggg taggggggaa atgccccct atttttgtc tagccatcct gccacgttg 1380
acagggtagc aatttcgaca cgatagggtt ctctctctg ccgtta 1426

```

<210> SEQ ID NO 71

<211> LENGTH: 1468

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 71, Example
71: designer nirA-promoter-controlled NADPH-dependent Alcohol
Dehydrogenase DNA construct (1468 bp)

```

<400> SEQUENCE: 71

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacia 240
gggcaacaac catggcgtac ccagacacct ttgaaggatt tgccgtcact gacactgcaa 300
aatggteccac aaccaagaag atagaattca ccccaaaaag gttccaggaa catgatatcg 360
atgtcaagat ccatgcctgt ggtatctgag ggagtgatgt tcacactgtt tgcgggggat 420
gggcaaaaacc agaccttccc gtgatcccag gacatgagat cgttggtag gttgtagag 480
tgggcccaaa agtgaagggg tttgaaattg ggcaaagagt tgggtgttga gctcaagttt 540
gggctgtct agagtgcgac acatgcaagg ataacaacga aacgtactgt cctcaatggg 600
tggaactta caatgccact tatcctgatg gtgacaaggc atgggggtgt tattcctctc 660
acatcagagt ccacgatcac tttgtattcc ctattcctga tgaacttcca actaatgctg 720
tgcccccaat gttgtgagct ggtatcacca cgtactctcc gttgtaaga aatggagctg 780
gtccaggaaa gaaggtgggt atcatcggaa ttggagggtt gggacatttt gccatcatgt 840
gggctagggc tcttggttgc gaagtgtaca cgtttcttag aacacatagc aaggaagctg 900
atgctaagaa attgggaact gaccatttta ttgcgacgtg ggaggacaaa gactgggcca 960
agaagattgg cagaaagctg gactttatca tttcgtgtgg aaattcggcc acgaaactttg 1020
atatggatgg ttacctcagt gtgctgaagg ttcattgtaa actcatttcc gtcggccttc 1080
cagaggagcc attcacgctg tctgctggaa gctttatcaa gaacggttgc tacttgggat 1140
cgtcccactt ggggaacaga caggagatgc ttgatagct gaaacttctg gctgataagg 1200
gcattgggtc ttggtatgag gagctcccaa tctctgagga agggctgaag gaaggactgg 1260
agagatgcca caacaatgac gtttaagtata ggttcacct gaccggttac gataaggcat 1320
tcaaatagta aggctgagat cttcttcagt gcattgtagt tgaatgaagg gttagggggg 1380
aaatgcccc ctatTTTTTg tctagccatc ctgccacgtt tgacagggtg gcaatttcca 1440
cacgataggg ttctctcttc tgccgtta 1468

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<210> SEQ ID NO 72
<211> LENGTH: 1555
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 72, Example
72: designer nirA-promoter-controlled NADH-dependent Butanol
Dehydrogenase DNA construct (1555 bp)

```

```

<400> SEQUENCE: 72
agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagtty ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgcaggga agcgcgcaaa atggcgagca catcatcgcg gttcaatggt 300
ttgaaacggc cgaactcgcc aaacgccatc gctttatcgg ccatcagctc caagttttcc 360
tcgccgatgc catagtccgc aagcccgagc ggcgccccga ggctcgacca gaactcgcgc 420
agccgctcga tgcctcaag cgccacatcc cgctccgatt tgcccgcggg atcgacatca 480
aagacgcgca ccgagctg ggcgaaacgg ctgacatctt catcgagcac atgcttcatc 540
cagttcggga aaaaatcgc caatccccgc gcgtgcggga tgtcatacac ggcggaacg 600
gcatgctcaa tattgtgctg cgcccagtc cgcgggagc ccatttgcaa gaagccgttt 660
aaggcgatcg tgcccagta catgatcgtc tcgcgagct cgtagttttc caaatcgttg 720
atcagtttgg gcgcccgttc gatcacggtt ttcaacacgc cttcacacat ccggtcttgc 780
aatggcgtgt tcggcgtatg gtggaatat tgctcaaaca catgcgacat catatcgaca 840
atgccgtaaa ccgatggtc tttcggaacg gtcacatgat acgtcggatc caaaatcgaa 900
aattgcggga acgtaaacgg gctgccccag ccgtatcttt ctttcgtctc ccagtttctg 960
atcacccaac cagaattcat ctccgaccgc gtcgcccga gcgtcaagac gacgccaac 1020
ggcagggcgc cggtagcggg cgcttttttc gtgataaact cccacggatc gccatcgaat 1080
ttcgcgccgg cggcgatcgc tttcgtgcag tcgatgagc tcgcccggcc aaccgcccgc 1140
aaaaattcga cgcttcccg cttgcaaatg tccaccctt ttcttacggt cgagacgcgc 1200
gggttcgggt cgacgcctgg cagttcgatg acctcggcgc caatattccc caatatcttc 1260
atgacttctc catacaatcc gtttcgtttg atgctgccgc cgccatacac gacgagcact 1320
ttcttgccgt agcggcgac ttcttctttg agacgttcga gctgcccttt tccgaaaatc 1380
agtttcgtcg ggttgcgga aataaactct tgcattaagg ctgagatctt cttcagtgca 1440
ttgtagttga atgaagggtt aggggggaaa tgcccccta tttttgtct agccatctg 1500
ccacgtttga cagggtagca atttcgacac gatagggttc tctcttctgc cgtta 1555

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<210> SEQ ID NO 73
<211> LENGTH: 1558
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 73, Example
73: designer nirA-promoter-controlled NADPH-dependent butanol
dehydrogenase DNA construct (1558 bp)

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<400> SEQUENCE: 73

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agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcggtt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgaaaatc cacatcactg ccgtaatatg cacaagtata taatttttcc 300
atagtttcat cattaatctc ccttggattt gatcctgtgc atggatctaa aacagcatta 360
tgagctataa atttaagatt agctttaatc tcattctcat ctattccata ctctttcatt 420
gatgaaggta tatttaattc cttattaaac ttatttatta aatctattag gtcactgtgt 480
aattctcttt cactttctcc ctttaatcca atatgtcttg caatgttagc atatctattt 540
tcacaagcct ttctattata tttaattaca tatggcaaga agatagcatt agcacatcca 600
tgtggtatat ggaatacagc ccctacctta tgagccatag aatgtactat tcctaaaagg 660
gcattagaga aggccattcc tgctaaacat tgagcctcat gcatttctcc cctagcttcc 720
atatctccct tatatgaatt tactaagtc atattaacca tttcaattgc ctttaagct 780
aaaggatctg taaagtttga tcttaaaact gcagtataag cctcaatagc atgagttaag 840
gcatccattc ctgtgtgagc tactaaactc tctggcatag tttctgctaa gctaggatca 900
acaatagcta tatctggagt tatttcaaaa tctgctaaag gatatttaat cttagcctta 960
taatcagtta ttactgagaa ggcagttacc tctgtagcag ttccagaagt tgatggaata 1020
gctacaaaact ttgcttttct tctaagctta ggtaatccaa aaggaaacaat ggccttttca 1080
aaagtaaaat caggatactc gtagaaaatc cacatagcct ttgctgcac aataggtgaa 1140
cctcctccta tggaaaactat ccagctctga ttaaattcct ccatttctt tgcacccttc 1200
ataacagttt ctactgatgg atctgttctc actccttcaa aaaccttgtt ttccatatta 1260
gcttccttta aataacttaa aaccttatct aaaaagccaa atcttttcat tgatccgcca 1320
ccaataacta taaaggcctt tttaccttct aaactcttta atacttctaa ggaatctttt 1380
ccatgatata tgtcccttgg taaagtaaat cttgccatta aggctgagat cttcttcagt 1440
gcattgtagt tgaatgaagg gttagggggg aaatgcccc ctattttttg tctagccatc 1500
ctgccacggt tgacagggta gcaatttcga cacgataggg ttctctcttc tgcggtta 1558

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<210> SEQ ID NO 74

<211> LENGTH: 3646

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 74, Example
74: designer nirA-promoter-controlled Phosphoenolpyruvate
Carboxylase DNA construct (3646 bp)

```

<400> SEQUENCE: 74

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcggtt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catggccggc gttccgcaag cccgccgga tgcccaggat ggagagcagc 300
agcgcctcct ccaggccggc gcgctcgggg tcgccttccg gcgaggcccg gtaccggggc 360
agcagctcca cctgcaggta gctgatgggg tcgacgtacg ggttgcgcaa ggcgctctgc 420

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cgcgccagca	cggggtggtg	ggccagaagc	ggcccgccga	aggtctcttc	caagagcgcc	480
cggttccgca	ccatggcctc	ctcgagcccg	gggaagaacc	ggttcgccag	gggcccgggc	540
accagccgca	ggtactccct	ggccacggcc	aggtcggcct	tggccagggc	cagggcccg	600
ccgtccagca	ccgagcggaa	gaagggccag	cgggcaaaca	tcgctggcg	aaggtcccgc	660
ggcaccgccc	caagaccttc	ggccagccc	taccagcccg	gcagcagaag	acgcacctgg	720
gtccaggcca	tcaccagg	gatggcccgc	agatcctgga	cgcgccgggc	cggcccgtg	780
cgggccaccg	ggcggaagc	gatctcag	gcgccgatct	cccggatcgg	ggtgaagtgc	840
tcgaagaact	cgaagaacc	gggctccc	agcagctggc	ggtaggcttc	cgcgaccgg	900
gcggcgccc	ggtccatggc	ctcccgccag	gccgcggga	cgggggaacc	gcccggggcg	960
agccctggc	gcccgcgcc	cggcctgctc	ggcgcggtcc	cggccccgcc	ggcgggggcg	1020
ggttctccg	gcgaaagcac	cgcctcttg	gcctgggcca	gcgtgtccc	ggcgccgcc	1080
agaaggaagt	ggttagaggag	ctgctccag	ttgcgcagg	ccagctcgg	gtgagcgtag	1140
cggctcccca	gcgctctcc	ctgctcgg	agccgcagg	ggcgcccac	ggtgcccggc	1200
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cgaccgtgga	agaagacac	cggcacgccc	gccgcccgg	ccaccgcgc	gatgccctcc	1320
tgggcccgt	agagggcca	gttgccc	aggtagccc	cgtccttct	ggagtcggag	1380
tagccgatca	tcacctcgca	gccgcgcgg	cgcgggct	ggacgccgaa	gacggggttc	1440
tgcagcagct	gctccatggc	gcggggccc	gcctccaggt	cggccaggg	ctcgaacagg	1500
gggaccacgt	caaagggcaa	ggggtggcg	gggcgtaca	gccccacctc	ccgcgccagg	1560
acgaacacct	ccagcacgct	ggcgggggc	cggcagccgc	tgatgatgta	agcccccg	1620
tcttccag	cccgcagagc	atccagggcc	acggccagct	cgcggctgc	gggcccgtag	1680
cccaccggcg	ccagcggcc	gggcgaagcc	agctcccgg	tcagcacgc	ctcccggcc	1740
gggcatcca	ggccaggtta	ctcccctg	gccatgaccc	caccggcttc	cagcagctcc	1800
gccaccgccc	gcccgtgggc	ctcgcatgc	tcccgcaggt	cagggggcgc	catggcctcg	1860
caaagacct	gggcccgcca	gcgcagggga	cgcaccagg	tggccgcgc	ctgcccagc	1920
ccggcatccc	gcagcccctc	ttccacctg	aagagcagg	gatccaggg	ggtggaacc	1980
ggggctccg	gcccctcgc	ggccgggggt	tcccggcg	tccccttgg	tggcgcgcc	2040
gcggcgggc	gggaaggccg	ttcgccgct	gggtccgctg	gaacggcg	ccctccagg	2100
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ggccggggag	gcggtgccc	tgcctcgact	gcagcgtcc	gccggtggg	tacagccggt	2220
gcctcggctg	gagccgcgc	ccggcgggc	gtctcgctcc	acacctcttc	cggcaccgcc	2280
cttgcgccc	agtgcgctg	cagccggtag	gcgaaccgcc	ggtaggctc	gcccgggaa	2340
cggccggcg	ccgcccggc	cagggggccc	acggcctctg	cgggcaagga	gggcagggga	2400
ggcagggaa	cccgttcttc	cgccacggac	agatcccgc	tcagggcg	caggcctcc	2460
gcgtacttct	gggcatctc	ggcccgcgc	taggcctgg	cccaggcgg	gaacctccgg	2520
gtcaciaaag	ggttgccgct	ccggtcacca	ccgatccagc	tccggaagg	cagggcggg	2580
ggaagaaccg	gcccgcgcc	gtagcggcg	gccaccgcc	cctcgaggc	ctccatgagc	2640
cgggcaaccg	cctcccacag	ggtggtggc	aggtagtaga	ggccgcgc	gaacctcctc	2700

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tccaccgagg gggggccgg gcgcagctcg cgggtgggcc agagcagggt gaaccgggcc 2760
accacctcgt ccaggteccc ctgcgccgcg tocagccggg ccaggggcctg gttgagctgc 2820
agcaggtggt ggcgcagggt gcgcgccgcg gtctcggctg gatgggcccgt gaaggtcagc 2880
tccagccggg ccgaggcgag gagggcacc acgtcgtcga attccatccc ttgggcctga 2940
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tcgcgccggc gggtcaccgc caccgggtgc cgctcctccg ccaggttgac cagatggaaa 3060
taggtcgaaa agggccggat cagcccttcg gccgcggcca ccgaaagccc gccgatctcg 3120
ggccgcagcg cctgcggggc ggctcgtcc cccgggtgct gccggagggtg cttggtgtgg 3180
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cccaggcccc gtcccaggag gtcgacctcc cgcttgagaa ggggggtacag ctctcttcc 3300
ggccgcccac ccggcggggg aacggatccc gccggggcag gcaacgcctc gccggacgag 3360
ccggccgccc caggggcggt cccctgccg ccgccccggg cctggcggtc ctgaccgtcc 3420
ttcgggtccc ggccgtcctt caggteccgg ctccccttcc ccgcccga gccggcccct 3480
gccggccggt caccctgcc gctcactaag gctgagatct tcttcagtgc attgtagttg 3540
aatgaagggt taggggggaa atgccccct atttttgtc tagccatcct gccacgtttg 3600
acagggtagc aatttcgaca cgatagggtt ctctcttctg ccgtta 3646

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<210> SEQ ID NO 75

<211> LENGTH: 1591

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 75, Example
75: designer nirA-promoter-controlled Aspartate Aminotransferase
DNA construct (1591 bp)

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<400> SEQUENCE: 75

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agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcggggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgcgagaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgagaatt tcggaaagat caaaaaaggc gccggcgagt ccataagaa 300
aattagtgcc atttgcgag gaagctgtaa aaaaaggtaa aaaaatctat tatttaaca 360
taggtcaacc tgatatacca acaccttcaa tttattttca gtatgaggaa aaacacagac 420
cccagattgt agcttataca cactcagcag gtcttctgcc actgaggag gcttttaca 480
aatattacgc cagattcgat atagatgttt tcccggatga aatcatcgtt accaatgggt 540
gaagtgaagc tgcctgttc gctatgacag tcgtggcgga tccgggagat gaaatacttg 600
ttctggagcc gttctatgcc aattaacgtg gatttgctgc tcaacttggg atcaatcttg 660
ttccagttag aactgcgccg gaggatggat atcaaatacc aaagatgggt gattttctgg 720
agaaaatcag tcacaggact aaagcaatca ttttttcaa tccctgcaat cctacggggg 780
ctgtttacga tgaaaaaaa cttgaagtta ttgctgaagt tgctttgaag agagatttgt 840
ttgtgatttc agacgagggt tatagagaat tcacttttga tggttttagg gccatatcta 900
tgatgagttt ttctaattt tctgacaaa gtcattgtgt tgacagtatt tctaaaagat 960
atagtgcctg cggcgccggg attggtacat ttattaccaa gaataaagat atatatcaag 1020

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cagctatgaa actggctcag gcgagactct gtctctgctat gacatctcaa tacggacta 1080
ttggctcttc gacgcttgac gatttgtatt attcacaat gagaaaagag tatgaaatga 1140
gaagagatgt tgtttacgaa gaacttcagc gcattgatgg agcagtcttc aagaaacctc 1200
acggggcttt ttatatctcg gtgaaacttc caatcgacaa ttctgaagat tttgtgaaat 1260
ttatgttgac ggagtatgag gttgaaggaa aaacgacaat ggtggcacct ctcaagtggat 1320
tttatgtaac accatctacg gggatgagtg agatcagaat agcgtatggt ctggaacgcg 1380
aacaactgag ggatgcagtt gcaattttga cttcaggttt gaaaacttac atagagagaa 1440
gaaaaaata ataaggctga gatctctctc agtgctattg agttgaaatga agggtaggg 1500
gggaaatgcc cccctatctt ttgtctagcc atcctgccac gtttgacagg gtagcaattt 1560
cgacacgata gggttctctc ttctgccgtt a 1591

```

<210> SEQ ID NO 76

<211> LENGTH: 1588

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 76, Example
76: designer nirA-promoter-controlled Aspartate Kinase DNA
construct (1588 bp) SEQUENCE: 76

```

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtagtcc gaaaacttga cagagttctt tcaacaaatc ttctgcttta 300
tcttcatgta caaggcatga tatctttatt tcagaagttg ttatcatttc tggctctatg 360
tttttcttcc tcaaaacttc aaaaaatctt gctgcaaacac ctctgtgata tttcattcct 420
aaacctatta ttgatacctt agaaaacccc cctgtgatct taatttcaca atcaagatct 480
ctcattgcct tctgtacgtc aaccgagtta ctttcaacaa ttgtgaaaga tagattaatg 540
tctcctgagt tactcaccia cgatatcata tcaacgtaa agccttttcc agcaggttct 600
ctaaatcagt cggcggttgc tttagaatct ttcaagctat aaacgctgac ctttacctga 660
tttttctcta tcgtcgcgcc tgtaacaacg ggtcgcctca gccattccgg aagttttccc 720
atcaccacag ttccctcctc atttgaaaac gaagaagcgc aataaattgg tacgctgtat 780
ttttttgcta tctcaacact tcgagaatgt agaaccctgg caccaagtgc ggagaattcc 840
aacatttcat cgtatgtaat ataagacagt ttttttgctt ggggaaagat cctgggatct 900
gttgtatata tgccagcgac gtcgctgtat atttcgcaga ttgtccaag ttttgcggca 960
atagcaacag cagaagtatc tgatcctcct cttccaagag ttgtcagttc atcgttttcg 1020
tttattccct gaaaccctgt aacaagtaaa acatcgttat gaaaagccag cgatcttaat 1080
tttcgggtcat ctatatcttt tatccttgcc gaattaaaat cgctgggtgt cagaatccga 1140
gcctgaaaag cattcaaaaga tacagcttcc atacctatc tgtcaaggta tatggatagc 1200
aaagcagcgg atatttgctt gccacaggat aacaacatat ccagttctct tggattgggt 1260
ttttcagaca gccttcttgc aagaaaaacc aatttgtctg ttgttttccc cattgcagag 1320
acaacaacga ttaacttttt cccatttttt actgttttca ctattttctc ggtaattttt 1380

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ttgattcttt ctatgctggc aagcgatgag ccaccatatt tttgtacaac aagtttcaga 1440
aaaaactacta aggctgagat cttcttcagt gcattgtagt tgaatgaagg gttagggggg 1500
aatgccccct ctattttttg tctagccatc ctgccacggt tgacagggta gcaatttcga 1560
cacgataggg ttctctcttc tgccgtta 1588

```

```

<210> SEQ ID NO 77
<211> LENGTH: 1411
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 77, Example
77: designer nirA-promoter-controlled Aspartate-Semialdehyde
Dehydrogenase DNA construct (1411 bp)

```

```

<400> SEQUENCE: 77

```

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtcccct gaaaatatta taaatctctg cattgagaac tgcacacccg 300
gcagcccctc tgattgtatt atgaacaaga gcaacgaacc caaactgttt gttcgaatac 360
tgtttcaacc tgccaacact gacactcatt ccaccaccaa gatctctgtg aaacttcggc 420
tgcggagaat caactcctgt caaataaaaa acagggtttt cagggtgcggg tggaaagatac 480
attcctttaa gtggttgaaa atcttcaaaa gctctcacia tttcacttaa agtggccttt 540
tctgagttt tgatagttat agatagcata tgaccgtcaa caacggctac cctggtgcac 600
tgtgcgaaaa tctcgagatt cgccgggtat atttttccat ctttcaatct gccaaagtatt 660
ttttttgatt cgggtcatgat tttttcttcc tcatttttta tgaacggcac aacattgtca 720
attatatcca tagatggtag accgggatat cccgcgccag agatagcctg cattgtgaca 780
acatttgctt cttctatacc gaatctatcc attatggggt taagaacctat tgtaaaacct 840
atcgttgagc aattagggtt ggtgattatt tttccttttc tcttttgagt ttcggttatt 900
ttcaaatgat ccagattaac ttcagggata attagaggta catcctcgtc cattctatgg 960
cttgccgcat ttgagaaaaa aatgtatcct gcgtttgcaa attcctcctc aatttcgcct 1020
gcaacatctg aaggcaaaag agaaaacaca taatcacaat ctatatcagg tgtgcatttt 1080
tttaaaacca tatctcctgc tttttcggct acaggaacat tcaagcgcca ttgaactgct 1140
tctctgtact tttttcccgc agaattatca gaagctgcca gagctgttat ctcaaaaaat 1200
ggatgatttg aaagcaattg aacaaatctc tgtccaacaa gcctctgtgc accaagaata 1260
gctaccttca ttaaggctga gatcttcttc agtgcattgt agttgaatga aggggttaggg 1320
gggaaatgcc ccctattttt ttgtctagcc atcctgccac gtttgacagg gtagcaattt 1380
cgacacgata gggttctctc ttctgccgtt a 1411

```

```

<210> SEQ ID NO 78
<211> LENGTH: 1684
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 78, Example
78: designer nirA-promoter-controlled Homoserine Dehydrogenase DNA
construct (1684 bp)

```

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<400> SEQUENCE: 78

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg      60
accagagttg ttcagttcac aggtagataa tgtcgcggtt cttgatagtt agcaataaat     120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat     180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa     240
gggcaacaac catgaacttt actatcttcg ccttcacccc ggatgacggt gcaaatctct     300
gccactatac tcattttctc caacaccatc agggcatctc gcaggtcaga ttcaggaact     360
ctatgggtca ctaagaccag ctgggogaac tcctcgccgc ttgtctctcg cagcacggta     420
gctatgctca gctgatggct gccaaaaacc ccggcgatgg ccgccaatac cccagggcgg     480
tccttaacca gcatccgaat atagaacttg ctctccactt tgtcgatagg aataaatctcc     540
ttggcttcaa aacaagtaca gccagcatg ccggtgggtac catgactgag gttatggcat     600
atctccatta tgtctccacc caccgcgcta gcagtgaggc tctgccctgc cctcttcca     660
aagaacatga cttctccacc cgcgtcccgc ttcacgaata cggcgttgta aacccccttt     720
accgcggcca gaggggtggt cataggtatg aaagccgggt ggaccggggc ttgcaccggg     780
ccgtcgattt cctgcccgat agccaggagc ttcaccacat agcccaattc ataaccgtac     840
ttgatattca aagggcttaa acgggtaatt ccttcaacgt aaacgttttc gaacgtaacc     900
cggctgttga aggcgatcga agccaggatg gcaattttac gagcagcatc atagccttct     960
acgtctgaag taggatcagc ttcagcatac cccagttcct gcgcctcttt caaagccctc    1020
gaaaactcta ggcttcctc gctcatctta gtaaggatgt agttggttgt cccgttaaca    1080
atccccatta cttctttgat tcgattggca cctagcgaat gcttcaaagg ataaattaag    1140
gggatgcccc caccaacact ggcttcaaaa aagaagtcca ccttgttctc ttcagctgcg    1200
gccagcagtt cctgcccgtg gaccgctatt aaatctttat tggcagtcac cagttctctg    1260
cctttgcgta acgcctgcaa aataaaggtt cgagccggtt cgatgcctcc tatgagttct    1320
acaacaacac ttatatggtc atcatccagt atgtctttga tatcggcgca aaggacgtct    1380
tcgcttaaac ctagactcaa aaccttctcc gggctttttt cgaggattcg cttgatcgcg    1440
actcctgtgc cggtaacggag cgaaataaca tcccggtttg aggctaaaag cttgaccaca    1500
ccggatccaa ccgttcocaa acccaagagg ccaatattaa ccaactaaggc tgagatcttc    1560
ttcagtgcat tgtagttaa tgaagggta ggggggaaat gccccctat tttttgtcta    1620
gccatctgc caggtttgac agggtagcaa tttcgacacg atagggttct ctcttctgcc    1680
gtta                                             1684

```

<210> SEQ ID NO 79

<211> LENGTH: 1237

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 79, Example
79: designer nirA-promoter-controlled Homoserine Kinase DNA
construct (1237 bp)

```

<400> SEQUENCE: 79

```

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg      60
accagagttg ttcagttcac aggtagataa tgtcgcggtt cttgatagtt agcaataaat     120

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acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgaaggtc ctggtagcgg ccacaacgac caatctcgga gcgggattcg 300
acgtctttgg actcgcgctg gatcttttca acgaagtgga gttttcttcc gatacaaaaag 360
agacaacat agaaagtact ggaaaatacg cttcggattt gaaggaccac aatctgtttt 420
tcgaagtctt caggttcttt gagagaaaaa cgggtacag agttccgcca gtcaggatca 480
agcagacatg caacatccct gtatcgagcg gtcttgatc gagcgcgct gtgatcgtcg 540
cggcactcca cattgcgaac gaaggaacgg gcagaaatct ttcacgggaa gatcttatga 600
aactcgctgt ggagctggaa ggacacccctg acaacgttgt acccgcttcc acagggggggc 660
ttgtggtctg ttatcaaaaac ggaagtcac ttgattttga aaagttcgag atcgatcttt 720
ctctcacatt tttcgttcca aacttttcca tgtgcacgaa cgagatgaga aagatccttc 780
cggagaaggt ccctttcgaa gatgcggtct tcaacataaa gaattcatgc cagttccttg 840
caaagatcgc agctggaaaag atcaaaagag ctctgaaata cgtgggagat cgacttcacc 900
agaactacag gataaacggc aataagaaga tgaagaggt tgtggaagcc atcttatcaa 960
aaaaatccga gtactggttt gtgagcggat ccggtccttc tgtttgttcc aatataaatg 1020
actttgaagg gattccctat ctcaaggacg ttctgaagct gaggggtaac aacaggggga 1080
tgatagtctc agaataagtaa ggctgagatc ttcttcagtg cattgtagtt gaatgaaggg 1140
ttagggggga aatgcccccc tattttttgt ctagccatcc tgccacgttt gacagggtag 1200
caatttcgac acgatagggt tctctcttct gccgtta 1237

```

<210> SEQ ID NO 80

<211> LENGTH: 1438

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 80, Example
80: a designer nirA-promoter-controlled Threonine Synthase DNA
construct (1438 bp)

<400> SEQUENCE: 80

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgaaattg ggaatactcg aaaagtacag agaatttctt cccgtaacag 300
acaaaacccc catgctctct ttgaatgaag ggaacactcc tctcataccc ctctgtaaca 360
tgagcagggg actcggaaata aacatctacg taaaatacga aggggccaat ccgacggggg 420
ccttcaaaga cagaggaatg gtcgttgccg tcgcaaaggc actggaagaa ggctcgaag 480
ccatcatgtg cgcttcaacg gggaacacct ccgcatccgc tgcccgctac gccgcaaggg 540
caggaataaa ggcatcggt ctgataccag aagggaagat cgcactcgga aagctggctc 600
aggcgatgat atacggcgcc gtggtgcttc aggtgagagg gaatttcgac aagtgtctgg 660
aactggtcaa ggagatcaca tccaaatcct ccatcacact cgtgaacagt atcaatccct 720
acagactcga aggtcagaaa acggccgctt ttgagatagt cgacgagctc ggagatgcac 780
cggactacca cttcatcccc gtgggaaaac cgggcaacat ctccgcttac tggatgggat 840

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acaaggagta ttatcagcat gggttctcca ccaaactgcc gaagatgatg ggattccagg 900
cggaaggggc cgccccata gttcgcggtc atcccataga aaaccggag acggtcgcca 960
ctgcaataag gatcggtaac cccgcgaact gggaaaaagc ggtccgggca cgcgatgaat 1020
cgggtggaga catcgcacatg gtgagcgacg aagaataact gcgcgcacag agactcttg 1080
ctcagaaaga agggatcttc tgtgagcccg catccgctgc atcgatagcg gggcttttga 1140
agaagcacag acaggaatc ttcaggggtg gagagatcgt tgtgtgtacc ctcacagggg 1200
acggtttgaa agatccgaac atcgtcatct cacagcttga accccaagg atcatagaag 1260
gaagagtaga agagattctg gaggtactcg acatatgata aggctgagat cttcttcagt 1320
gcattgtagt tgaatgaagg gttagggggg aaatgcccc ctattttttg tctagccatc 1380
ctgccacgtt tgacagggta gcaatttcga cacgataggg ttctctcttc tgccgtta 1438

```

<210> SEQ ID NO 81

<211> LENGTH: 1600

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 81, Example
81: designer nirA-promoter-controlled Threonine Ammonia-Lyase DNA
construct (1600 bp)

```

<400> SEQUENCE: 81

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgttgaca ttagcagaca ttgaacaagc gcgagcgaaa atgaaaggca 300
tcgtccatca aacgccgctt gagcattcgc aaacgttcag cggctgtctt ggcaatgatg 360
tatatatgaa actcgaaaat ttgcaaaaaa cgggctcgtt taaagtaaga ggttcattca 420
ataaaattat gtcgctcacg gaagaagaga gggcgcgcgg cgtcatcgcc gcttcggccg 480
gcaaccacgc ccaaggggct gcctatgcga gcggcatgct tcatattccg tgcacgatcg 540
tcatgccaaa aggcgcgcgg ctcagcaaaa ttgaagcgac gaaaagctac ggggcggaag 600
tcgtgctgta cggcgatgtg tttgacgagt ctttggaaata tgcgttagag ttgcagcgtg 660
aacgggggat gacgtttgtt catccgtttg acgacttggc ggtgatggcc ggccaagggg 720
cgatcggtt agagctgac gagcagcttc ccgacgtcga tgcgttctt tgtccagtcg 780
gcccggggg gttgcttgcg ggggtggcgc ttacgttaa acagctgaag ccgtcggttg 840
aagtgtacgg cgttgagtca tcggcttgcc ccggcatgac ggcggccata cgcataaac 900
agcccgcttc cattgccgca tcgaatacga tcgccgatgg gattgccgtg aaaaagccgg 960
gcaatattac gtaccaatac attgagcaat acgtcgatgg cgttgatgac gtggaagagg 1020
cgaaatttc gcggacgatg ctgtatgtgc tcgagcggaa caagctgttg atcgaagggg 1080
cggcagcttg tccgctggcg gcattgttgt atcaaaagct gccgtttcgc ggcaaaaaag 1140
tcgcccctt ttaagcggc ggcaacgtcg atgtgacgct catttcccgc atcatcgagc 1200
ggggctcgt cgaagccggc cgattcgtta cgtttacaac ggtcatctcc gacaagccgg 1260
gccagttgaa caagctgctg cgcattattg cggagcttga ggcaaacgtg atgtcgattc 1320

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atcatcagcg catcggcgcc aaagtgctgc caggtcaggc gaaattcac tttcgcctcg 1380
agacaaaaaa cgaagaccac attcagcaaa tctaccaagt gttgttgaag gaaggctacg 1440
atgtacagtt ttaccgatga taaggctgag atcttcttca gtgcattgta gttgaatgaa 1500
gggttagggg gaaatgccc ccctattttt tgtctagcca tcctgccacg tttgacaggg 1560
tagcaatttc gacacgatag ggttctctct tctgcccgtta 1600

```

```

<210> SEQ ID NO 82
<211> LENGTH: 2107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 82, Example
82: designer nirA-promoter-controlled Acetolactate Synthase DNA
construct (2107 bp)

```

```

<400> SEQUENCE: 82
agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatattctgta atacaaaaac tgcacgaga caagaaaaaa gtagcaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgttgaca aaagcaacaa aagaacaaaa atcccttggtg aaaaacagag 300
ggggggagct tgttgttgat tgcttagtgg agcaaggtgt cacacatgta tttggcattc 360
caggtgcaaa aattgatgag gtatttgacg ctttacaaga taaaggacct gaaattatcg 420
ttgcccggca cgaacaaaaa gcagcattca tggcccgaag agtcggcctg ttaactggaa 480
aacccgggag cgtgttagtc acatcaggac cgggtgcctc taacttgcca acaggcctgc 540
tgacagcgaa cactgaagga gaccctgtcg ttgcgcttgc tggaaacgtg atccgtgcag 600
atcgtttaaa acggacacat caatctttgg ataatgcggc gctattccag ccgattacaa 660
aatacagtgt agaagttcaa gatgtaaaaa atataccgga agctgttaca aatgcattta 720
ggatagcgtc agcagggcag gctggggccg cttttgtgag ctttccgcaa gatgttgtga 780
atgaagtcac aaatacgaag aacgtgcgtg ctgttgacgc gccaaaactc ggtcctgcag 840
cagatgatgc aatcagtgag gccatagcaa aaatccaaac agcaaaaactt cctgtcgttt 900
tggtcggcat gaaagggcga agaccggaag caattaaagc ggttcgcaag cttttgaaaa 960
aggttcagct tccatttgtt gaaacatac aagctgcccg tacctttct agagatttag 1020
aggatcaata ttttgccgt atcggtttgt tccgcaacca gcctggcgat ttactgctag 1080
agcaggcaga tgttgttctg acgatcggct atgaccgat tgaatatgat ccgaaattct 1140
ggaatatcaa tggagaccg acaattatcc atttagacga gattatcgt gacattgatc 1200
atgcttacca gcctgatctt gaattgatcg gtgacattcc gtccacgac aatcatatcg 1260
aacacgatgc tgtgaaagtg gaatttcag agcgtgagca gaaaatcctt tctgatttaa 1320
aacaatatat gcatgaaggt gagcaggtgc ctgcagattg gaaatcagac agagcgcacc 1380
ctcttgaat cgttaaagag ttgcgtaatg cagtcgatga tcatgttaca gtaacttgcg 1440
atatcgggtc gcacgccatt tggatgtcac gttatttccg cagctacgag ccgttaacat 1500
taatgatcag taacggtatg caaacactcg gcgttgccct tccttgggca atcggcgtct 1560
cattggtgaa accgggagaa aaagtgggtt ctgtctctgg tgacggcggg ttcttattct 1620
cagcaatgga attagagaca gcagttcgac taaaagcacc aattgtacac attgtatgga 1680

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acgacagcac atatgacatg gttgcattcc agcaattgaa aaaatataac cgtacatctg 1740
cggtcgattt cggaaatata gatatcgtag aatatgcgga aagcttcgga gcaactggct 1800
tgcgcgtaga atcaccagac cagctggcag atgttctgcg tcaaggcatg aacgctgaag 1860
gtcctgtcat catcgatgac cgggttgact acagtataaa cattaattta gcaagtgaca 1920
agcttccgaa agaattcggg gaactcatga aaacgaaagc tctctagtaa ggctgagatc 1980
ttcttcagtg cattgtagtt gaatgaaggg ttagggggga aatgcccccc tattttttgt 2040
ctagccatcc tgccacgttt gacagggtag caatttcgac acgatagggt tctctcttct 2100
gccgtta 2107

```

<210> SEQ ID NO 83

<211> LENGTH: 1405

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 83, Example
83: designer nirA-promoter-controlled Ketol-Acid Reductoisomerase
DNA construct (1405 bp)

```

<400> SEQUENCE: 83

```

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcggggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgttttac ctctttaagc caaggcatca tcgccctaag ttccttacct 300
actttttcta tcaggtgctc ctgcccttc ctccgcaagg cactgaagac cggacggccc 360
acttggaaat cgagcaagag ctccccggca aaagtccat cctggatggc agccaaaacc 420
tttttcactc cagccctggt gttttcgttt attatgcgog gacctaccgt gaggtcggcg 480
tactcagcgg tatcactgac cgagtaccgc ataaggccga taccgccttc gtatataagg 540
tctactatga gcttaagctc gtgcaggcac tcgaaatagg ctatctccgg ctggtatcct 600
gcctctacca aggtatcaaa accggcttta ataagttcag tgaccccccc acagagcaca 660
cattgttctc cgaataggtc ggtctcgttt tctcttttaa aagtagtagc gatgacacct 720
gcacgggtac agccgatacc tttagcatag gctaaccocg tttccaagge tttccccgta 780
tggtcattat gtacggctat gagccccggg actcccactc cttgcctgta catacgctg 840
accagatgac caggactcct aggcgctacc atgaagacat cgacggaagg cggaggcaca 900
atttgcccga aatgtatggt gaaccatga gaaaaacca acgcatcgcc ttcgttaagg 960
taaggttcga tcttttcccg gtaaaccttg gcctggatat catccggcac caaaatctgg 1020
attatctggg cagctcgggc cgcttcatct accggtaaag gcgtaagccc gtcggctaca 1080
acctgattcc actccgcagt ggtaaaatcg tctccggct tacgcaaccc taccaccact 1140
tcgagaccac tgtcgtgaag gttctgggcc tgagcgtgtc cctggctgcc ataaccgatc 1200
acggcaatgg tcttgctttt aagcaggtea aggtttgcat cagcatcata atacatccta 1260
gccattaagg ctgagatcct cttcagtgca ttgtagttga atgaaggggt aggggggaaa 1320
tgcccccta tttttgtct agccatcctg ccacgtttga cagggtagca atttcgacac 1380
gatagggttc tctcttctgc cgtta 1405

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<210> SEQ ID NO 84
<211> LENGTH: 2056
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 84, Example
      84: designer nirA-promoter-controlled Dihydroxy-Acid Dehydratase
      DNA construct (2056 bp)

<400> SEQUENCE: 84
agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg    60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat    120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat    180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa    240
gggcaacaac catggggctt cctgaagatt gcccccttgc tcgccgactg tacgaagaat    300
gcgtaccttc tcaggtaatc gctgtccact tctttcacca gaggcgtgaa ctctttcatt    360
cttctttcga actcttcgct tgagatcaag agattcaggg ttctcttttc aaaatctatc    420
tcgatgaggt ccccgctctt cacgatacct ataggaccgc cttctgccgc ttctggagaa    480
acgtgacctc tcacggcacc gtgcgatcca cccgagaacc taccgtctgt gatgagagcc    540
acgtcctccg caaggcccat ccccacgatg gcggaggtgg gtgagagcat ctctctcatc    600
ccgggaccgc ccttcggccc ttcgtagcgg atcacaacca catctccttt tttgatcttt    660
ccagatagaa tggcttttgt cgcctcttct ccgcttctca agacgacggc cgggccaacg    720
tggtgcatca tcttctcggg aacaccgag agtttgcaa cgcctccttc tggagcgagg    780
ttcccgaaga ggataccgag tccgcctctc ttgtggtacg gattatcgaa gggcctgatc    840
acatcttcat tcaggatctt agcctctctg acgagatctc caatctttct caaatagatg    900
gtcatggcgt ctctcttcaa aagaccattt tcctggagac gtttcatcac agcgtagata    960
ccaccagcat cgtcgagatc ctggatgtgg tacggaccaa cgggagagat gttgcagatg    1020
tgaggaatct tcctgctgag ttcgtcaaag agctttatat cgaaatctat tccaaaactc    1080
tcggctatcg ccttcaaatg cagaactgtg ttcgtggaac ctcccgttgc gaggtccacc    1140
atgacagcgt tcatgaaaga gtccagagtg acgatatccc ttggttttac atctcttttc    1200
acgagttcca caacgagcat ccccgttctc ttcgccattc tcaacctctt cgcgtggagc    1260
gccggtacag tcccatctcc cctcggtgca attccgagag cttccgccag agagttcatc    1320
gtgttcgctg tgaacaatcc agcacacgaa ccggcaccgg gacacgcgag gtctttctatc    1380
gctttgagcg tttcttcatc gacttttccc actttgtatc caccaaccgc ttcgaagacg    1440
gtgatgagat cgatgtctct gccgtttag cgacctgcca gcattgggacc gccggatatac    1500
agaacggagc ggatgttcaa tcttcccatt gccatcatca tgccgggtgt gatcttctgc    1560
cagttgggga cgaagaccaa accatcgaag gggaaaccgc ttgcaacgat ctctatggag    1620
tccgctatga gttccctcga gggcaaggaa aacttcatcc ccctgtgata cattgtctatt    1680
ccgtcacaga tcccgatcgt tggaaagacg aagggaactc ccccggccat tctcacaccg    1740
gctttcaccg cttcaacgac cttgtcaagg tggacatggc cgggaatgat ctctgtccac    1800
gaggacacta tgccgatgaa aggccttcgc atttcgtcgt ccgttattcc gagcgttttc    1860
aaaagtgate tatggggagc cctttcgaga cctttcttta tcacatcact cctcattaag    1920
gctgagatct tcttcagtgc attgtagttg aatgaagggt taggggggaa atgccccct    1980

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atTTTTtGtc tagccatcct gccacgtttg acagggtagc aatttcgaca cgatagggtt 2040
ctctcttctg ccgтта 2056

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<210> SEQ ID NO 85
<211> LENGTH: 1360
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 85, Example
      85: designer nirA-promoter-controlled 2-Methylbutyraldehyde
      Reductase DNA construct (1360 bp)

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<400> SEQUENCE: 85

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agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catggcttct gtaaatgact actttgagaa cgccaagacg acgtacttta 300
ctttgagatc gggtgacaag atccccgctg ttggattggg tacttgcaa tcaccacca 360
acgagactaa agaggcagtc aagtacgctt tgcagcacgg ttaccgtcac atcgatgctg 420
ccgcattta tggaacgaa gacgaggttg gtgacggtat caaggagagt ggaatccctc 480
gtgacaaaat ctgggtcaca tctaagctct ggtgcaatgc tcatgctccc gaggctgtcc 540
ccaaggcttt ggagaagacc ttgctgagc tgaacttga ttacctgac cttacctca 600
tccactggcc tatttcttgg aagaccggcg atgacttggg tccaaggac aaggacggca 660
acaccatcac tgtcgaatc cccctcgagg acacctgaa ggctatggag ggtcttctga 720
agtccggcaa ggtgaagaac attggtattt ccaatttcaa caacgaagag ttggatcgta 780
tttgaaggt tgcagagatt cctctgccc tccacaaat ggaactcat ccttacttga 840
agcagacgga gttcattgag aagcacaaga agcttggcat tcacgtcacc gttactcgc 900
ctttggccaa ccaaaatgct cttaacggca atgccgttcc caagtgtatt gagcacaaga 960
ctctgtcga cattgccaag accaaggggt agggcgtcac tggtgccaac attgctattt 1020
cttgggcagt caagcgcggt acttcggtta ttcctaagtc tgttcatgcc aacagaatta 1080
agagcaactt cctcgttgtt cccctgactg atgacgagat gaaggccatc gataacattg 1140
gtgtcagcaa gcgtttcaat tggagcaaag tttctgcaa tgagaattgt ttctacggtc 1200
ttgagatgg tctcagtaa taaggctgag atcttcttca gtgcattgta gttgaatgaa 1260
gggttagggg gaaaatgcc cctattttt tgtctagcca tcctgccacg tttgacaggg 1320
tagcaatttc gacacgatag ggttctctct tctgccgтта 1360

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<210> SEQ ID NO 86
<211> LENGTH: 1420
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 86, Example
      86: designer nirA-promoter-controlled 3-Methylbutanal Reductase
      DNA construct (1420 bp)

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<400> SEQUENCE: 86

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agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60

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accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgtcagtt ttcgtttcag gtgctaacgg gttcattgcc caacacattg 300
tcgatactct gttgaaggaa gactataagg tcatcggttc tgccagaagt caagaaaagg 360
ccgagaatth aacggaggcc tttggtaaca acccaaaatt ctccatggaa gttgtcccag 420
acatatctaa gctggacgca tttgaccatg ttttccaaaa gcacggcaag gatatacaaga 480
tagttctaca tacggcctct ccattctgct ttgatatac tgacagtga cgcgatttat 540
taattctctg tgtgaacggt gttaaaggaa ttctccactc aattaaanaa tacgccgctg 600
attctgtaga acgtgtagtt ctacactctt cttatgcagc tgtgttcgat atggcaaaag 660
aaaaagataa gtctttaaca tttaacgaag aatcctggaa cccagctacc tgggagagtt 720
gccaaagtga cccagttaac gcctaactgt gttctaagaa gtttgcgtaa aaagcagctt 780
gggaatttct agaggagaat agagactctg taaaattcga attaactgcc gttaacccag 840
tttacgtttt tggctccgaa atgtttgaca aagatgtgaa aaaacacttg aacacatctt 900
gcgaactcgt caacagcttg atgcatttat caccagagga caagataccg gaactatttg 960
gtggatacat tgatgttcgt gatgttgcaa aggcctcattt agttgccttc caaaagaggg 1020
aaacaattgg tcaaaagacta atcgtatcgg aggccagatt tactatgcag gatgttctcg 1080
atatacttaa cgaagacttc cctgttctaa aaggcaatat tccagtgggg aaaccaggtt 1140
ctggtgctac ccataacacc cttggtgcta ctcttgataa taaaagaggt aagaaattgt 1200
taggtttcaa gttcaggaac ttgaaagaga ccattgacga cactgcctcc caaattttaa 1260
aatttgaggg cagaataata taaggctgag atcttcttca gtgcattgta gttgaatgaa 1320
gggttagggg ggaatgccc ccctattttt tgtctagcca tctgcccacg tttgacaggg 1380
tagcaatttc gacacgatag ggttctctct tctgccgta 1420

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<210> SEQ ID NO 87

<211> LENGTH: 1540

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 87, Example
87: designer nirA-promoter-controlled 3-Ketothiolase DNA construct
(1540 bp)

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<400> SEQUENCE: 87

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agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgcgtgaa gcggctcattg tcgaagcggg caggacgccg gtcggcaagc 300
ggaaacggcgt cttccgggac gttcatccgg tccatttggc cgcggtggtg ctcgatgaag 360
tcgtgcgccg gcccggcatg gacaaagggg cgggtggaaga catcgtcatg ggctgcgtga 420
cgccggtcgc cgaacaaggg tacaacatcg gccggctggc ggcgcttgag gccggattcc 480
cgatcgaagt gccggcagtg caaatcaacc gaatgtgcgg ctcggggcag caggcgattc 540
atctgcgccg ccaggaaatc cgctccggcg atatggatgt cacgatgcc gccggggtcg 600

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aaagcatgac gaaagtgcg attttaagcg atggcaacga gcggacgatt ccgccgtcgc 660
tgcatgaaaa atacgaattc atccaccaag gcgtctcggc tgagcggatc gccaaaaaat 720
acggcctaac gcgcgaggag cttgacgcct acgcgtacga aagccatcaa cgcgccttgg 780
cggccttgcg cgaagggaa tttcgcgcgg aaatcgtccc ggtgaaaggg cttgaccgcg 840
atggccgcga aatccttgtc accgatgatg aagggccgcg ggccgacaca tcgccggaag 900
cgctcgcgcg gctcaagcgg gtgtttcaag aagacggtct catcaccgct ggcaatgcga 960
gccaaatgag cgacggggcg gccgctgtgc ttttgatgga acgggaggcg gcgaggcggg 1020
tcggactgaa gccgaaagcg cgcattgtcg cgcaaacggt cgtcggctcc gacccgacgt 1080
atatgctcga tggcgtcatt ccggcgacga ggcaagtgct gaaaaaagcc ggcctctcga 1140
tcgatgacat cgacctcatt gaaatcaacg aagcgttcgc cccggtcgtg ctgcctggc 1200
aaaaagaaat cggcgtcctc cttgagaagg tgaatgtcaa cggcggcgcc attgcgcttg 1260
gccatcgcct cggcgccacc ggtgcgaagc tcatgacgtc gcttggtcat gaacttgaa 1320
ggcgcggcgg ccgctatggg ctattgacga tttgcatcgg ccacgggatg gcgacggcca 1380
cgatcatcga gcgggagtaa taaggctgag atcttcttca gtgcattgta gttgaaatgaa 1440
gggttagggg ggaaatgccc ccctatttt tgtctagcca tcctgccacg tttgacaggg 1500
tagcaatttc gacacgatag ggttctctct tctgccgta 1540

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<210> SEQ ID NO 88

<211> LENGTH: 1231

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 88, Example
88: designer nirA-promoter-controlled 3-Hydroxyacyl-CoA
Dehydrogenase DNA construct (1231 bp)

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<400> SEQUENCE: 88

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agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgatactt aaagaaacct tctccggtct tacgcccag cttccctgca 300
cggaccatct taaccaagag cgggcaagga cgatacttgt catcgccaaa ctgcgatgc 360
aaagtctgca tgatagccaa acatatatct agcccaatca tgtctgctaa agcgagggga 420
cccataggat gccctgctcc gagcttcatg gaggtatcca cgtcctcggg gcttgcaacc 480
ccctccatga cggcatacat accttcgttc agcattgaa taagcaggcg gttgacaaca 540
aagccaggag cttegttgat ctcaaccggg gtcttgccca gcttaattga aagatccttg 600
atggtattaa aagtttctcg gctagtagaa gccctttgta taatctcgat aagcttcatt 660
gccggtaccg ggttgaagaa atgcatgcct attaccctgt ctgcccgett ggttgetgct 720
cctatctcgg ttatgctcag agctgatgtg ttagaagcca ggatacatc aggcttgca 780
atctcgtcca gttccttgaa aatcgctttt ttgatgtcca tattctcgat agcagcttca 840
attaccacat ccacatcttt ggccgcagcc atgtcgaccg taccgctaat cctggccatc 900
accggttct tgcatctcgc gctcatcttg cccttttcga ccattttgct gagacccttg 960

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tcgatgcctt ttataccatt gtoaacaac tcttgtttaa taccacgtac gattacttcg 1020
aaccagcctt gagcagcgac ttgaacaac ccagctccca tagtacctgc gectaaaacc 1080
attatattca ttaaggctga gatctcttc agtgcttctg agttgaatga aggggttagg 1140
gggaaatgcc ccctatttt ttgtctagcc atcctgccac gtttgacagg gtagcaattt 1200
cgacacgata gggttctctc ttctgccgtt a 1231

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<210> SEQ ID NO 89
<211> LENGTH: 1162
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 89, Example
89: designer nirA-promoter-controlled Enoyl-CoA Dehydratase DNA
construct (1162 bp)

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<400> SEQUENCE: 89
agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatattctga atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacia 240
gggcaacaac catgacgggt cgactggaat acgatggcgg gttcgcgcac ctgacgctca 300
gcccgcgcga ggtcctgaat gcgctcagtt tcgagctgct cgcagagttg agccggggcg 360
ttgcccggct cgcgcaatcc gatgcgcgcg ccctgatcgt cacgggcgag ggcgacaagg 420
cgttctgcgc cggcgcggac attcccagac tgatgaatcg gccgctcatg caagagctcg 480
aaggggcccg gaaaggccag gcggtgttca gccggatcgc cgagctgaag attccgtctg 540
tcgcccgtcat ccagggttat gccttcggcg gcgggctgga gcttgcctcg gcatgcacat 600
tccgcgttgc cactgatcgc gcccgcatgg ggctgccca ggtcaagctc ggctgatcc 660
cgggttatgg cggaaacgag cgtctgccga ggctgatcgg cgagggggcg gcaactcgacc 720
tgatcatgtc cggccgcacg atagacggcg gggaaagcga gcgaatcggc ctgggtcaatc 780
gcatagacia cgaggggacg cccctggaga tcggcaagcg gttctggag ccttatctca 840
agcacagtct ctgcgccttg tattttgccc gcgagggcgt gcagagggga ggcgggtgctg 900
ccattgcgga tggcctgcgc atcgagcggg atctttccac gctggcttac cggagccagg 960
atgcggccga ggggctgcgc gcttttgagg aaaaacggcc cgcgtcttcc aaggactgct 1020
gataaggctg agatcttctt cagtgcattg tagttgaatg aaggggttagg ggggaaatgc 1080
ccccctattt ttgtctagc catcctgccca cgtttgacag ggtagcaatt tcgacacgat 1140
agggttctct cttctgccgt ta 1162

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<210> SEQ ID NO 90
<211> LENGTH: 1561
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 90, Example
90: designer nirA-promoter-controlled 2-Enoyl-CoA Reductase DNA
construct (1561 bp)

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```

<400> SEQUENCE: 90
agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120

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acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat	180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa	240
gggcaacaac catggccggc gcgcagcagg atcttgccgc tgcgtccggg cttgtcgctg	300
gccgcggcgg ccttgccggc atcgtgcagg tcgaacaccg cttccaccgg cagcgcagg	360
ctgccatcga gcgcggcggg gagcagttcg cggatcatgc ggcgcttgtc ctgggccttg	420
gtggcctgca tcacctgtct gccccagaag ccacgcacgg tggcctgctt gaagatcaca	480
tcgcgcgtgg atatatcgag cggtcgccg gtcacgcagc caaaggaaat cagctcgccg	540
ccttcggcca gcaaggccat cagctcaccg gctgcattgc cggccaccga atcgatggcg	600
cgcacgatgg gcgcacgcc ggccagcggc cgcacctgt cctgccagcc tgettgcgca	660
gtggagattg cgttgccgat gccagcggc ttcagctcgt ccacgcgggc gtcgcccgc	720
accaggttga tcacgttgat gccgcgtgcg gcggcgagca tcgccaccgt cttgccgacc	780
gcaccgttgg cgggtgtctg cacgatccag tcgccctggt tcacctgcag gaattcgatc	840
agcatcagcg cgtcagcgg catggcgatc aactggcaac cacgctcgtc gtccaggcca	900
tccggcaacg gcaccacgcc ggaggcgtcg gcaaggaagt actcggccca ggccctatgc	960
acaccggcgg cgaccacgcg ctggccaacc tgcaagccct cgacaccctc acccagcgc	1020
tcgatgacac ccgccgcttc gctgcgccg atggctggca gttccggctt gtagccgtaa	1080
ttgccgcgca cgggtccacag gtcattggta tggatcgcg cgcccgcat cgcaacgcgc	1140
acctggccct tgccctggct gcggcgtggg cgctcgccca gttcgagcac cttggccgga	1200
tcgccgaatt gggatggat ggctgcgcg atggaggtct cctgccgggc acgctcttgc	1260
tcgcagcgc ccgatcgtt tgaaagggtg cgcgatgcta tcggcagggc tgcaagggaag	1320
ggatgaagcg aacggaaact ctgtgtgaag ttgttggcgt gcgcgcgtag tgacgatgct	1380
ctgtgcagc gccggaggac tgcgtgcagg ccgaccctca ttaaggctga gatcttcttc	1440
agtgcattgt agttgaatga agggttaggg gggaaatgcc cccctatctt ttgtctagcc	1500
atctgccac gtttgacagg gtagcaattt cgacacgata gggttctctc ttctgccgtt	1560
a	1561

<210> SEQ ID NO 91

<211> LENGTH: 1747

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 91, Example 91: designer nirA-promoter-controlled Acyl-CoA Reductase DNA construct (1747 bp)

<400> SEQUENCE: 91

agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg	60
accagagttg ttcagttcac aggtagataa tgcgcggggt cttgatagtt agcaataaat	120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat	180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa	240
gggcaacaac catgtagttg tctactaact acgtaagtca tttcctgcaa attgtgcatt	300
ccatcatgag aagttctcgg ataattgtct ggcatttccc ctggcttagt gactatgctt	360
acaccaagta acgtcatttt ttcaataaat tcgtggctcg caccactata ccccatagtt	420

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tgaaggaatt gctttaaatt ttottcaaca taatcatgta cttcatcttt attttcatat	480
gggcaaacaa atataagtct attaaagcat ctatcaatat ccttttttcc aggcattctg	540
ttgottaaaa tcacagtata gtccgcatca cacgatgcaa acacctttgc cggtttctcc	600
tcatcaacac tatattttaa taaacaatac tgcgggtcct gtattgattt catagaactc	660
caaggactca gatatgcctt tgggaaaacc tctgtaagct ctttcaagct ttctgctaca	720
gtttctgcaa gaatattaat gtctattttt ttattggcaa ataccatcct aggagacaaa	780
caggcttttt gctcccaaca tatcacatca cttagcaatac cttttgcaat agtcttaata	840
tcttctactt tatctataac ttcaaaaacta attttagcac catgcattat taaatgagaa	900
ttatattttg cacataactc tgccattatc cttcctgaat attctccacc ccaatgtata	960
acacaatcca tttctctcac gacagtctca tatatatcag aacattcact actaaagtat	1020
aaaaagata gtctatcttt tataacttggg tcaagctgta ccaaaacttc atagaaagca	1080
tacgcaaaa atgggttcac agcagaaaacc tttactaaat tacagttcct tgataataac	1140
cccataccta tacttctcgg aacaactaca aatgcatttc cagaaatatt atgaaacatc	1200
acacctcttg gctgtctatg cacagctcca taacttgttg gaaccaatt atctagtata	1260
tcaatgttac caagttcttc tttaatgatt atctcaagat tttctcttaa aagcattctc	1320
atactatttt caagttcata tgttacaagt tcttcaactt gattcaatat gttagctaat	1380
ctttctatat gtacttttggg gtatcctcta tcaagccaca atcttccaca cctatccaaa	1440
agatcaattg tatcctgcac tgatattgca tgactcttac ttttactttt tctaagtctt	1500
tttatttctt caattacctg atctctactt gagtaagtta attctaattc caggccattt	1560
atattcttta ttaaaacatt actttcaca acagtttctc tcttcattaa ggetgagatc	1620
ttcttcagt cttgtgattt gaatgaaggg ttagggggga aatgcccccc tattttttgt	1680
ctagccatcc tgccacgttt gacagggtag caatttcgac acgatagggt tctctcttct	1740
gccgtta	1747

<210> SEQ ID NO 92

<211> LENGTH: 1450

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 92, Example
92: designer nirA-promoter-controlled Hexanol dehydronase DNA
construct (1450 bp)

<400> SEQUENCE: 92

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg	60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat	120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat	180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa	240
gggcaacaac catggaactc gacctcgacg gtcccggggt tggtaagtgt ctgatcaagt	300
acaccgccgc ggggttctgc cattcggacc tgcacttgac cgacggggac ctaccgccgc	360
gtatccaat cgtcgggggg cacgaggggt caggcatcat cgaggacgtc ggacctgggg	420
tcaccaaggt caaacaggc gatcacgttg tttgcagctt catcccgaac tgcggaacct	480
gtcggtagct cgcaccgga cgtccaacc tctcgatat gggcgcacc atcctcgaag	540
ggtgcatgcc cgacggcagt taccggttcc acagtaacgg cctggatttc ggtgcatgt	600

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gcatgctcgg cacattctcc gaacgcgcaa ctatctccca gcattcgggtg gtcaggatcg 660
acgactggct gccgctcgag accgcgggtg tegtccggctg cggcgtgccg actggctggg 720
gcacctccgt ctatgcgggc ggggttcggt cgggtgacac caccgtcacc tatggcgtcg 780
gcgccctggg agtcaacgcc gtccaaggcg cggtgagtgc gggcgcgaag tacatcgtgg 840
tcgtcgatcc ggttgcgctc aaacgcgaca ccgcgctcaa gttcggcgcc acccacgcgt 900
tcgcccagcg cgccaccgcc ggggccaagg tcgacgaact gacctgggga cagggtgccg 960
atcaggcgct gatcctggtc ggcaccgtcg acgaggacgt ggtctcggcg gcgactcggg 1020
tgatcggtaa gggaggcacc gtcgtgatca ccggactggc ggaccagca aagctcacgg 1080
tgacggttc gggaaacggac ctgacgctta acgagaagac aatcaagggc acgttgctcg 1140
gctcgtccaa tccgcaatac gacatcgtac ggctgctcgg tctctacgac gccggccagc 1200
taaaactcga cgatctgac accacccgat acacgctcga ccaggtaaac cagggtacc 1260
aggatctcgc agacggcaag aacatccgcg gcgtgatcat ccacgcctga taaggctgag 1320
atcttcttca gtgcattgta gttgaatgaa gggtagggg ggaatgccc ccctatcttt 1380
tgtctagcca tctcgccacg tttgacaggg tagcaatttc gacacgatag ggttctctct 1440
tctgccgtta 1450

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<210> SEQ ID NO 93

<211> LENGTH: 1074

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 93, Example
93: designer nirA-promoter-controlled Octanol Dehydrogenase DNA
construct (1074 bp)

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<400> SEQUENCE: 93

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agaaaatctg gcaccacacc tgacccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgttggga ggccaagaag ccgctggtga ttgaggacat tgagggtggc 300
ccacctcagg cttggcaggt tcgcatcaag attacagcca ctggcgtttg ccacacggat 360
tctttttcgt tgagcggctc tgatcctgag ggtctcttc ccgtggtcct tggccatgag 420
ggcgccggca tcgtggagag cgttggcgag ggcgtaacca actttaaggc cggcgatcat 480
gtcattgccc tctacatacc ccagtgcaat gagtgcaaat tctgcaagag cggcaagaca 540
aatctctgcc agaagattcg cctcaccag ggcgctggtg tcatgcccaa tggatcctcc 600
cgcttgctgt gcaagggtca gcagctgttc cattcatgg gcacctcaac ttcgcccag 660
tacgcggtgg tggccgacat atcggtgacc aaaatcaacg agtcggctcc attggagaag 720
gtgtgccttc tgggctgtgg catttccacg ggctatggtg ccgcctttaa caccttagg 780
tggaaactcg cagcacttgc gccgtctggg gtctgggtgc tgttgactg gcagtggttc 840
tgggctgcaa gaaggctggc gccgccaagg tctacggcat cgacatcaat cctccaaat 900
tcgagctggc caggaagttc ggcttccacc actttaaggc tgagatcttc ttcagtgcac 960
tgtagttgaa tgaagggtta ggggggaaat gccccctat tttttgtcta gccatcctgc 1020

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cacgtttgac agggtagcaa ttctgacacg atagggttct ctcttctgcc gtta 1074

<210> SEQ ID NO 94
<211> LENGTH: 1096
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 94, Example
94: designer nirA-promoter-controlled Short Chain Alcohol
Dehydrogenase DNA construct (1096 bp)

<400> SEQUENCE: 94

agaaaatctg gcaccacacc tgaccccat cgagagactc cgaacgtggc aaatggaggg 60
accagagttg ttcagttcac aggtagataa tgtcgcgggt cttgatagtt agcaataaat 120
acagtttcag aatatctgta atacaaaaac tgtatcgaga caagaaaaaa gtagcaaaat 180
ttacaaatgt tcatgattca tctggctaaa ttggatgttc aactgaccca ttgaagacaa 240
gggcaacaac catgaaggtt gccgtaatta ctggggcatc ccgtggaatc ggggaagcta 300
tagcaaaagg ccttgctgaa gatggatatt cccttgcctt aggggctaga agtgttgata 360
ggttagagaa gattgccaag gaactcagcg aaaaacatgg ggtggaggtta ttttacgact 420
acctcgatgt atcaaaacca gaaagcgttg aagagtttgc aaggaaaacg ctagctcact 480
ttggagatgt ggacgttgtt gtggccaatg cggggccttg ttactttggt aggcttgaag 540
agcttacaga agagcagttc cacgaaatga ttgaagtaaa ccttttggga gtttgagaaa 600
caataaaagc tttcttaaac tccttaaacg ggactggagg agtggctatt gttgttactt 660
cagatgtttc tgcaaggcta cttccatacg gtggaggtta tgtggcaact aaatgggctg 720
caagagcatt ggtaaggacc ttccagattg agaatccaga tgtgaggttc ttcgagctaa 780
gacctggagc agtagataca tttttggag ggagcaaacg tgggaagcca aaggagcaag 840
ggtattttaa acctgaggaa gttgctgagg cagtaaaata cctcctaaga cttccaaagg 900
atgttagggg tgaggaatta atgttgcgct caatttatca aaaacctgag tattgataag 960
gctgagatct tcttcagtgc attgtagttg aatgaagggt taggggggaa atgccccct 1020
attttttgc tagccatcct gccacgtttg acagggtagc aatttcgaca cgatagggtt 1080
ctctcttctg ccgtta 1096

<210> SEQ ID NO 95
<211> LENGTH: 1438
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 95, Example
95: designer Synechococcus sp. strain PCC 7942 nirA-promoter-
controlled NADPH-dependent Glyceraldehyde-3-Phosphate-
Dehydrogenase DNA construct (1438 bp)

<400> SEQUENCE: 95

agaaaatctg gcaccacacc cttcttgacg aacatgcatg atttcaaaaa agttgtagtt 60
tctgttacca attgcaatc gagaactgcc taatctgccg agtatatgat gtcaacgaat 120
attgcaatta atggaatggg tagaattgga agaatggtgc taagaatagc actaaagaat 180
gaagcattga atgtagtgtc catcaatgct agctatcctc ctgaaacaat tgcacattta 240
attaattatg acacaacaca tgggagatag gataaaagag tagaacctat tgaagtgga 300
attcgagtgg aaggccatga tattaatta gtgtctgata gaaaccaga aaatttacc 360

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tggaaagatt tagaaataga tatcgtcatt gaagcgaccg gtaaatttaa ccatgggtgat	420
aaagctaagg cacatattca agcaggagct aaaaaagtgt tattgacagg accatcaaaa	480
ggcgaaaaag tacagatggg ggtaaagggt gttaacgatc aagacttaga tacagataca	540
tatgacatat ttagtaatgc gtcgtgtact acgaattgta tcggaccagt tgcaaaagtt	600
ttaaagata gttttggcat tgaaaatggc ttaatgacaa cggtagatgc aattacaaat	660
gatcaaaata ataatagata tccgcataaa gatttgagaa gagcgcgttc ttgtggggaa	720
agtattatac caacatcaac aggtgctgct aaagcattaa aagaagtat gccagaattg	780
aatggcaaac tacatggcat agcacttcgt gtgccaactc aaaatgtatc attagttgat	840
ttagtcattg atttaaaaca aaaagtgaca gtagatgaag ttaatcatgc atttagagat	900
gcaaaacttac aaggaattat tgatgttgaa gaggcccctc tagtttctaa ggactataat	960
acaaatcctc attcagcagt tatagatgct aaaaatacaa tggatcatggg agataataag	1020
gttaaagtta tagcctggta tgataacgaa tggggatatt ctaatagagt agttgaggta	1080
gcaaatcaac ttggagaact aattaataa taatagtgat cccggccgct actaaagcct	1140
gatttgtctt gatagctgct cctgcctttg ggcaggggct tttttctgct tgccattctt	1200
gaggatggcg gactctttcc cttttgctct acgcccatag atgcgatcgc agtctcccct	1260
gtccagcacg ttggagtgat tgggtggggc cagttagctt ggatgctggc accagcagcg	1320
caacagttgg ggatgctgct gcacgttcaa acacccaatg atcacgaccc agcagtagcg	1380
atcgcggatc aaaccgtatt agcagcagtt gctgacgcgg ttctctcttc tgccgtta	1438

<210> SEQ ID NO 96

<211> LENGTH: 1447

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 96, Example 96: designer *Synechococcus* sp. strain PCC 7942 nirA-promoter-controlled NAD-dependent Glyceraldehyde-3-Phosphate-Dehydrogenase DNA construct (1447 bp)

<400> SEQUENCE: 96

agaaatctg gcaccacacc cttctgacg aacatgcatg atttcaaaa agttgtagtt	60
tctgttacca attgcgaatc gagaactgcc taatctgccg agtatatgat ggcagtaaaa	120
gtagcaatta atggttttgg tagaattggg cgttttagcat tcagaagaat tcaagaagta	180
gaaggtcttg aagttgtage agtaaacgac ttaacagatg acgacatggt agcgcattta	240
ttaaaatag acactatgca aggtcgtttc acaggtgaag tagaggtagt tgatgggtgg	300
ttccgcgtaa atggtaaaga agttaaatac ttcagtgaac cagatgcaag caaattacct	360
tggaaagact taaatatcga tgtagtgtta gaatgtactg gtttctacac tgataaagat	420
aaagcacaag ctcatattga agcaggcgct aaaaaagtat taatctcagc accagctact	480
ggtgacttaa aaacaatcgt attcaacct aaccaccaag agttagacgg ttctgaaaca	540
gttgtttcag gtgcttcag tactacaac tcattagcac cagttgctaa agttttaaac	600
gatgactttg gtttagttga aggtttaatg actacaatc acgcttacac aggtgatcaa	660
aatacacaag acgcacctca cagaaaagg gacaaacgtc gtgctcgtgc agcggcagaa	720
aacatcatcc ctaactcaac aggtgctgct aaagctatcg gtaaagtat tcctgaaatc	780
gatggtaaat tagatgggtg tgacacaact gttctctgag ctacaggttc attaaactgaa	840

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ttaacagtag tattagaaaa acaagacgta acagttgaac aagttaacga agctatgaaa 900
aatgcttcaa acgaatcatt cggttacact gaagacgaaa tcgtttcttc agacgttgta 960
gggatgactt acggttcatt attcgacgct acacaaactc gtgtaatgtc agttggcgac 1020
cgteaattag ttaaagttgc agcttggtat gataacgaaa tgtcatatac tgcacaatta 1080
gttcgtacat tagcatactt agctgaactt tctaaataat aatagtgatc ccggccgcta 1140
ctaaagcctg atttgtcttg atagctgctc ctgcctttgg gcaggggctt ttttctgtct 1200
gccattcttg aggatggcgg actccttccc ttttgctcta cgcccatgaa tgcgatcgca 1260
gtctcccctg tccagcacgt tggagtgatt ggtggtgccc agttagcttg gatgctggca 1320
ccagcagcgc aacagttggg gatgtogctg cacgttcaaa cacccaatga tcaogacca 1380
gcagtagcga tcgcgatca aaccgtatta gcagcagttg ctgacgcggt tctctcttct 1440
gccgtta 1447

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<210> SEQ ID NO 97

<211> LENGTH: 2080

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 97, Example
97: designer Synechococcus sp. strain PCC 7942 nirA-promoter-
controlled 2-Keto Acid Decarboxylase DNA construct (2080 bp)

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<400> SEQUENCE: 97

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agaaaatctg gcaccacacc cttctgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcgaate gagaactgcc taatctgccg agtatatgat gtatacagta 120
ggagattacc tgtagaccg attacacgag ttgggaattg aagaaatctt tggagttcct 180
ggtgactata acttacaatt tttagatcaa attatctcac gcgaagatat gaaatggatt 240
ggaaatgcta atgaattaaa tgcttcttat atggctgatg gttatgctcg tactaaaaaa 300
gctgccgcat ttctcaccac atttggagtc ggcaattga gtgcgatcaa tggactggca 360
ggaagttagt ccgaaaatct accagtagta gaaattgttg gttcaccac tcaaaaagta 420
caaaatgacg gaaaatcttg ccatcataca ctacagatg gtgattttaa acactttatg 480
aagatgcatg aacctgttac agcagcgcgg actttactga cagcagaaaa tgcacatat 540
gaaattgacc gagtactctt tcaattacta aaagaaagaa aaccagtcta tattaactta 600
ccagtcgatg ttgctgcagc aaaagcagag aagcctgcat tatctttaga aaaagaaagc 660
tctacaacaa atacaactga acaagtgatt ttgagtaaga ttgaagaaag tttgaaaaat 720
gccccaaaac cagtagtgat tgcaggacac gaagtaatta gttttggttt agaaaaaacg 780
gtaactcagt ttgtttcaga aacaaaacta ccgattacga cactaaatct tggtaaaagt 840
gctgttgatg aatctttgcc ctcatcttta ggaatatata acgggaaact ttcagaaact 900
agtcttaaaa atttgtgga gtcccagac tttatcctaa tgcttgagtg gaagcttacg 960
gactcctcaa caggtgcatt cacacatcat ttatagatgaaa ataaaatgat ttcactaaac 1020
atagatgaag gaataatctt caataaagtg gtagaagatt ttgattttag agcagtggtt 1080
tcttctttat cagaattaaa aggaatagaa tatgaaggac aatatattga taagcaatat 1140
gaagaattta ttccatcaag tgctccctta tcacaagacc gtctatggca ggcagttgaa 1200
agtttgactc aaagcaatga aacaatcgtt gctgaacaag gaacctcatt ttttgagct 1260
tcaacaatct tcttaaaact aatagctcgt tttattggac aacctttatg gggttctatt 1320

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ggatatactt ttccagcggc tttaggaagc caaattgcgg ataagagag cagacacctt 1380
ttatttattg gtgatgggtc acttcaactt accgtacaag aattaggact atcaatcaga 1440
gaaaaactca atccaatttg ttttatcata aataatgatg gttatacagt tgaagagaa 1500
atccacggac ctactcaaag ttataacgac attccaatgt ggaattactc gaaattacca 1560
gaaacatttg gagcaacaga agatcgtgta gtatcaaaaa ttgttagaac agagaatgaa 1620
tttgtgtctg tcatgaaaga agcccaagca gatgtcaata gaatgtattg gatagaacta 1680
gttttggaag aagaagatgc gccaaaatta ctgaaaaaaaa tgggtaaatt atttgtctgag 1740
caaaaataat agtaatagt atccccggcg ctactaaagc ctgatttgtc ttgatagctg 1800
ctcctgcctt tgggcagggg cttttttctg tctgccattc ttgaggatgg cggactcttt 1860
cccttttgct ctacgcccat gaatgogac gcagctctcc ctgtccagca cgttgagtg 1920
attggtggty gccagttagc ttggatgctg gcaccagcag cgcaacagtt ggggatgctg 1980
ctgcacgttc aaacacccaa tgatcacgac ccagcagtag cgatcgcgga tcaaaccgta 2040
ttagcagcag ttgctgacgc ggttctctct tctgccgta 2080

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<210> SEQ ID NO 98

<211> LENGTH: 1603

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 98, Example
98: designer Synechococcus sp. strain PCC 7942 nirA-promoter-
controlled NADH-dependent Butanol Dehydrogenase DNA construct
(1603 bp)

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<400> SEQUENCE: 98

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agaaaatctg gcaccacacc cttctgacg aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgcaatc gagaactgcc taatctgccg agtatatgat gtaagattt 120
acactaccaa gagatattta tttcggagaa aacactttag aaactttaa aactttaa 180
ggtaagaaa ctataattgt tgttggagga ggatcaatga aaaaatttg ttccttcaa 240
aaagttgaag aatatctaaa agaagcagga atggaaataa aattaataga aggtgttga 300
ccagatccat cagttgaaac cgttatgaaa ggtgcagaaa tcatgagaga ttttgagcct 360
gattggatag tatccatagg tggaggatca ccaatagatg ctgctaaagc tatgtggata 420
ttctatgaat acccagaatt tacttttgag caagctgttg ttccttttg aataccagat 480
ttaagacaaa aagctaaatt tgttgctata ccatctaaa gtggaacagc tacagaagtt 540
actgctttt cagttataac tgattacaaa gctaagataa aatccctt agctgatttt 600
aatttaacac cagatgtagc tattatagat ccagctcttg ctcaacaat gcctgcaaaa 660
ttaacagctc atacaggtat ggatgcttta actcatgcaa tagaagctta ttagcagga 720
ttaagatcat atttctcaga tcctctgca atgcaagcta tagttatgac aaaagataat 780
ttaataaaat cctatgaagg agataaagaa gcaagagatg aaatgcatat agctcaatgt 840
ttagcaggaa tggcattctc aaatgcgcta cttggaatta ctcatagtat ggcacataag 900
acaggagcag tattccacat tcctcatggt tgtgcaaatg ctatattcct tccttatgta 960
atagatttta ataagaaaac atgtaaagat agatatgcaa ctatagctaa aactttaggt 1020
ttagcaggaa atactgatga tgaattagta gatgcattaa cttctatgat acaagaaatg 1080
aataagaaaa tggatatacc actaaactta aaagaatatg gagtaacaga agaagatttt 1140

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aatgaaaact tagatttcat agcacataat gcagtgtag atgcatgtac tggatcaaat 1200
ccaagaccta taactgaaga agaaatgaaa aaagtattca aatgcacatt tactggagag 1260
aaagtaatt ttaataata gtgatccgg cgcctactaa agcctgattt gtcttgatag 1320
ctgctctgc ctttggcag gggcttttt ctgtctgcca ttcttgagga tggcggactc 1380
ttccctttt gctctacgcc catgaatgcg atcgcagtct cccctgtcca gcacgttgga 1440
gtgattgggt gtggccagtt agcctggatg ctggcaccag cagcgcaaca gttggggatg 1500
tcgctgcacg ttcaaacacc caatgatcac gaccagcag tagcgcgcg ggatcaaac 1560
gtattagcag cagttgctga cgcggttctc tcttctgccg tta 1603

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<210> SEQ ID NO 99

<211> LENGTH: 1654

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 99, Example
99: designer Synechococcus sp. strain PCC 7942 nirA-promoter-
controlled NADPH-dependent Butanol Dehydrogenase DNA construct
(1654 bp)

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<400> SEQUENCE: 99

```

agaaaatctg gcaccacacc cttctgcag aacatgcatg atttcaaaa agttgtagtt 60
tctgttacca attgccaatc gagaactgcc taatctgccg agtatatgct agaaatcaac 120
ttctgtatcg taataacagc acttaagcag tttttccatt tcttctacgc ttggtgtctt 180
tgggttagaa cctgtgcagg catcgcctat ggcattaact gcaatgtcat gtaacctctc 240
caggaataca ttttcaggca caaatccctg tgcaaccgga tagctgtctg caccgtaatt 300
ttaaagcag tgccgtatata taagttcatc attcatctta cggagataac cgattaatga 360
agctaccttt tcatcaaggc ctgctccgcc aagtcccatg aaatcagcaa tttcaccata 420
acgcttctta gcctgttcat cctttgcggt aaatgcaatt acctagggga gatacattgc 480
attcgcagca ccgtgaatga tgtgtgcgcc gtaatcggca aatgccgcac ctgttttatg 540
cgccattgaa tgtacaatac caagaagtgc attagaaaat gccattcctg cgagacattg 600
tgcattatgc attgaatctc tttttccat atcaccgta tatgaaccga caaggctctt 660
ttgaatcatt ttaattgcat ggagtgccaa tgggtctgta aaatcacaat ttgcggtgga 720
tacatatgcc tcgatagcat gtgtcattgc atccatacct gtatgtgcca ccaatttttg 780
tggcatggtc tctgccagtt caggggtctac tattgcaaca tcaggtgta tttcaaaatc 840
ggctattgga tattttatc ctttttcata atctgtaata attgaaaaag cagttacctc 900
ggtagcgggt cctgaagtag aagatattgc acaaaaatgt gcttttttac gaagtgaagg 960
tatgccaaat actttacaca tatcctcaaa ggtaatatca ggatattcat atttaacca 1020
cattgcttta gccgcatcaa tcggagaacc tccgcctatt gcaacaatcc agtcaggttc 1080
aaactctgac atcgtcttgg cacctttcat aacggtttcc accgaagggt caggttcaat 1140
tccttcaaaa agtctgactt ccataccggc ttccttaaga tactgttctg cctgtcaag 1200
gaaacaaaaa cgtttcattg aacctccgcc aacacaaatc atggcttttt tgctttgaaa 1260
tgtcttaagt gcctctaagt caccctttcc atgatacaaa tctcttgga acgtaaatct 1320
tgccattaat agtgatcccg gccgtaacta aagcctgatt tgtcttgata gctgctcctg 1380
cctttgggca ggggcttttt tctgtctgcc attcttgagg atggcggact ctttcccttt 1440

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tgctctacgc ccatgaatgc gatcgcagtc tcccctgtcc agcacgttgg agtgattggt 1500
ggtggccagt tagcttggat gctggcacca gcagcgcaac agttggggat gtcgctgcac 1560
gttcaaacac ccaatgatca cgaccagca gtagegatcg cggatcaaac cgtattagca 1620
gcagttgctg acgcggttct ctcttctgcc gtta 1654

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<210> SEQ ID NO 100
<211> LENGTH: 1440
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 100, Example
100: designer Synechocystis sp. PCC 6803 nirA-promoter-controlled
NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase DNA
construct (1440 bp)

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<400> SEQUENCE: 100
atggtagtta aagttggtat taacggttcc ggctgatatc gacgtcttgc attccgccgt 60
attcaaaata tcgaagggtg tgaagtaact cgtatcaacg accttacaga tccaaatatg 120
cttgcaact tgttgaata cgatacaact caaggctggt ttgatggaac agttgaagtt 180
aaagaagggt gatttgaagt aaacggaac ttcattaaag tttctgtga acgtgatcca 240
gaaaacatcg actgggcaac tgatggggtt gaaatcgttc tggaagcaac tggtttcttt 300
gctaaaaaag aagcagctga aaaacactta catgtaacg gtgctaaaaa agttgttatac 360
acagctcctg gtggaacgca tgttaaaaca gttgttttca acactaacca cgacattctt 420
gacggtactg aacagttat ctacaggtgct tcatgtacta caaactgttt agtcctatg 480
gctaaagctc ttcacgatgc attcggattt caaaaaggct ttatgactac aatccacgct 540
tacactggtg accaaatgat ccttgacgga ccacaccgtg gtggtgacct tcgtcgtgca 600
cgcgctgggt ctgcaaatat cgttcctaac tcaactgggt ctgctaaagc tatcgtctct 660
gttatcccag aacttaacgg taaacttgac ggtgctgcac aacgtgttcc tgttccaact 720
ggatcagtaa ctgagttggt tgtaactctt gacaaaaacg tttctgtga cgaatcaac 780
gctgctatga aagctgcttc aaacgatagc ttcggttaca ctgaagatcc aatcgtttct 840
tcagatatcg taggcgtatc atacggttca ttgtttgacg caactcaaac taaagtaatg 900
gaagttgacg gatcccaatt ggttaaagtt gtatcatggt atgacaacga aatgtcttac 960
actgctcaac ttgtactgac tcttgagtac ttcgcaaaaa ttgctaaata atagtaatga 1020
gttacagttt tggcaattac taaaaaactg acttcaattc aatgtagcc cgtccccgctg 1080
ggttttttgt tgctttttca cagtactatc aggtaatcag caacacaata cggccctggt 1140
ctttggacag tttttgtata atgttgaccg catcctgacc ggatttttta tctaagtggg 1200
gaattgtcaa ttgtcaatta aagctaagtt ctactaatgt tttagaaggc attgtcgatt 1260
gaaaataaag gttgaatgga gaaaattttg agcctttgtc aaagataaaa atttatttca 1320
acagtttttt aactagccga accagagaat gaccagtggt cgctgacttt gctccccgag 1380
ttttgttaga aattaccctc aagaagtaat ctaataataa ggttctctct tctgccgtta 1440

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<210> SEQ ID NO 101
<211> LENGTH: 2182
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 101, Example

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101: designer *Synechocystis* sp. PCC 6803 nirA-promoter-controlled
2-Keto Acid Decarboxylase dehydrogenase DNA construct (2182 bp)

<400> SEQUENCE: 101

```

agaaaatctg gcaccacacc ctaaatgcgt aaactgcata tgccttggtt gagtgtaatt    60
tacgttacaa attttaacga aacgggaacc ctatattgat ctctacatga tgtatacagt    120
aggagattac ctggttagacc gattacacga gttgggaatt gaagaaatth ttggagtthc    180
tggtgactat aacttacaat ttttagatca aattatttca cggaagata tgaatggat    240
tggaaatgct aatgaattaa atgcttctta tatggctgat ggttatgctc gtactaaaaa    300
agctgccgca tttctcacca catttgagat cggcgaattg agtgcgatca atggactggc    360
aggaagtatt gccgaaaatt taccagtagt agaaattggt gggtcaccaa cttcaaaagt    420
acaaaatgac ggaatattg tccatcatac actagcagat ggtgatttta aacactttat    480
gaagatgcat gaacctgtta cagcagcgcg gactttactg acagcagaaa atgccacata    540
tgaattgac  cgagtacttt ctcaattact aaaagaaaga aaaccagtct atattaactt    600
accagtcgat gttgtgcgag caaaagcaga gaagcctgca ttatctttag aaaagaaag    660
ctctacaaca aatacaactg aacaagtgat tttgagtaag attgaagaaa gtttgaaaaa    720
tgccaaaaaa ccagtagtga ttgcaggaca cgaagtaatt agttttggtt tagaaaaaac    780
ggtaactcag tttgtttcag aaacaaaact accgattacg aactaaatt ttggtaaaag    840
tgctgttgat gaatctttgc cctcattttt aggaatatac aacgggaaac ttcagaaat    900
cagtcctaaa aattttgtgg agtccgcaga ctttatccta atgcttgag tgaagcttac    960
ggactcctca acaggtgcat tcacacatca tttagatgaa aataaaatga ttcactaaa   1020
catagatgaa ggaataatth tcaataaagt ggtagaagat tttgatttta gagcagtggt   1080
ttcttcttta tcagaattaa aaggaataga atatgaagga caatatattg ataagcaata   1140
tgaagaatth attccatcaa gtgctccctt atcacaagac cgtctatggc aggcagttga   1200
aagtttgact caaagcaatg aaacaatcgt tgctgaacaa ggaacctcat tttttggagc   1260
ttcaacaatt ttcttaaaat caaatagtcg ttttattgga caacctttat ggggttctat   1320
tggatatact tttccagcgg ctttaggaag ccaaattgag gataaagaga gcagacacct   1380
tttatttatt ggtgatggtt cacttcaact tacctgacaa gaattaggac tatcaatcag   1440
agaaaaactc aatccaatth gttttatcat aaataatgat ggttatacag ttgaaagaga   1500
aatccacgga cctactcaaa gttataacga cattccaatg tggaaatthc cgaatthacc   1560
agaaacatth ggagcaacag aagatcgtgt agtatcaaaa atgttagaa cagagaatga   1620
atthgtgtct gtcattgaaag aagcccaagc agatgtcaat agaatgtatt ggatagaact   1680
agttttggaa aaagaagatg cgcctaaaat actgaaaaaa atgggtaaat tatttgctga   1740
gcaaaataaa tagtagtaat gagttacagt tttggcaatt actaaaaaac tgacttcaat   1800
tcaatgttag cccgctcccg cgggtttttt gttgcttttt cacagtgact ataggtaatc   1860
agcaacacaa tacggccctg ttctttggac agtttttgta taatgttgac cgcactcctga   1920
ccggattttt tatctaagtg gggaaatgct aattgtcaat taaagctaaag ttctactaat   1980
gthttagaag gcattgtcga ttgaaaataa ggggttgatg gagaaatthc tgagcctthg   2040
tcaaagataa aaatthattt caacagthtt ttaactagcc gaaccagaga atgacctagc   2100
ggcgtgact  ttgctcccga gthtttgta gaaatthacc tcaagaagta atctaataat   2160

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aaggttctct cttctgccgt ta 2182

<210> SEQ ID NO 102
 <211> LENGTH: 1705
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 102, Example
 102: designer Synechocystis sp. PCC 6803 nirA-promoter-controlled
 NADH-dependent Butanol Dehydrogenase DNA construct (1705 bp)

<400> SEQUENCE: 102

```

agaaaatctg gcaccacacc ctaaatgcgt aaactgcata tgccttggt gagtgaatt    60
tacgttacia attttaacga aacgggaacc ctatattgat ctctacatga tgtcaagatt    120
tacctacca agagatatatt atttcggaga aaacacttta gaaacttta aaactttaaa    180
aggtaagaaa gctataattg ttggttgagg aggatcaatg aaaaaattg gtttccttca    240
aaaagttaa gaatatctaa aagaagcagg aatggaaata aattaatag aagggttga    300
accagatcca tcagttgaaa cggttatgaa aggtgcagaa atcatgagag attttgagcc    360
tgattggata gtatccatag gtggaggatc accaatagat gctgctaaag ctatgtggat    420
attctatgaa taccagaat ttacttttga gcaagctgtt gttccttttg gaataccaga    480
ttaaagacia aaagctaaat ttgttgctat accatctaca agtggaaacag ctacagaagt    540
tactgctttt tcagttataa ctgattacaa agctaagata aaatattcctt tagctgattt    600
taatttaaca ccagatgtag ctattataga tccagctctt gctcaaacia tgctgcaaaa    660
attaacagct catacaggtg tggatgcttt aactcatgca atagaagctt atgtagcagg    720
attaagatca tttttctcag atcctcttgc aatgcaagct atagttatga caaaagataa    780
ttaaataaaa tcctatgaag gagataaaga agcaagagat gaaatgcata tagctcaatg    840
tttagcagga atggcattct caaatgcgct acttggaaatt actcatagta tggcacataa    900
gacaggagca gtattccaca ttctctatgg ttgtgcaaat gctatattcc ttcttatgt    960
aatagatttt aataagaaaa catgtaaaga tagatatgca actatagcta aaactttagg    1020
tttagcagga aatactgatg atgaattagt agatgcatta acttctatga tacaagaaat    1080
gaataagaaa atggatatac cactaaactt aaaagaatat ggagtaacag aagaagattt    1140
taatgaaaac ttagatttca tagcacataa tgcagtggtg gatgcatgta ctggatcaaa    1200
tccaagacct ataactgaag aagaaatgaa aaaagtattc aaatgcacat ttactggaga    1260
gaaagttaat ttttaatagt aatgagttac agttttggca attactaaaa aactgacttc    1320
aattcaatgt tagcccgcct ccgcggtgtt tttgttgctt tttcacagtg actataggta    1380
atcagcaaca caatacggcc ctgttctttg gacagttttt gtataatggt gaccgcatcc    1440
tgaccggatt ttttatctaa gtggggaatt gtcaattgtc aattaaagct aagttctact    1500
aatgttttag aaggcattgt cgattgaaaa taagggttga atggagaaaa ttttgagcct    1560
ttgtcaaaga taaaaattta tttcaacagt ttttaacta gccgaaccag agaatgaccc    1620
agtggcgctg actttgctcc cgagtttttg ttagaaatta ccctcaagaa gtaatctaata    1680
aataagggtc tctcttctgc cgtta    1705

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<210> SEQ ID NO 103
 <211> LENGTH: 1756
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 103, Example
    103: designer Synechocystis sp. PCC 6803 nirA-promoter-controlled
    NADPH-dependent Butanol Dehydrogenase DNA construct (1756 bp)

<400> SEQUENCE: 103
agaaaatctg gcaccacacc ctaaatgcgt aaactgcata tgccttggtt gagtgaatt    60
tacgttacia attttaacga aacgggaacc ctatattgat ctctacatgc tagaaatcaa   120
cttctgtatc gtaataacag cacttaagca gtttttccat ttcttctacg cttggctgtc   180
ttgggttaga acctgtgcag gcacgcgcta tggcattaac tgcaatgtca tgtaacctct   240
ccaggaatac attttcagcg acaaatccct gtgcaaccgg atagctgtct gcaccgtaat   300
ttttaatgca gtgcggtata ttaagttcat cattcatctt acggagataa ccgattaatg   360
aagctacctt ttcacaaagg tctgtctcgc caagtcccat gaaatcagca atttcaccat   420
aacgcttctt agcctgttca tcctttgcgt taaatgcaat taccttaggg agatacattg   480
cattcgcagc accgtgaatg atgtgtgcgc cgtaatcggc aaatgccgca cctgttttat   540
gcgccattga atgtacaata ccaagaagtg cattagaaaa tgccattcct gcgagacatt   600
gtgcattatg cattgaatct cttttttcca taccaccggt atatgaaccg acaaggtctc   660
tttgaatcat ttaattgca tggagtgcca atgggtctgt aaaatcacia tttgcggtgg   720
atacatatgc ctcgatagca tgtgtcattg catccatacc tgtatgtgcc accaattttt   780
gtggcatggt ctctgccagt tcagggctca ctattgcaac atcaggtggt atttcaaaat   840
cggctattgg atattttatt cctttttcat aatctgtaat aattgaaaaa gcagttacct   900
cggtagcggg tcctgaagta gaagatattg cacaaaaatg tgctttttta cgaagtgaag   960
gtatgccaaa tactttcac atatcctcaa aggtaatata aggatattca tatttaatcc  1020
acattgcttt agccgcatca atcggagaac ctccgcctat tgcaacaatc cagtcagggt  1080
caaaactcga catcgctttg gcacctttca taacggtttc caccgaaggg tcaggttcaa  1140
ttcctcaaaa aagtctgact tccataccgg ctctcctaag atactgttct gcctgtcaa  1200
ggaaacccaa acgtttcatt gaacctccgc caacacaaat catggctttt ttgccttgaa  1260
atgtcttaag tgctctaat gcacccttc catgatacaa atctcttggg aacgtaaatc  1320
ttgccattag taatgagta cagttttggc aattactaaa aaactgactt caattcaatg  1380
ttagcccget cccgcggggt ttttgttget ttttcacagt gactataggt aatcagcaac  1440
acaatacggc cctgttcttt ggacagtttt tgtataatgt tgaccgcac ctagccggat  1500
tttttatcta agtggggaat tgtcaattgt caattaaagc taagttctac taatgtttta  1560
gaaggcattg tcgattgaaa ataagggttg aatggagaaa attttgagcc tttgtcaaag  1620
ataaaaattt atttcaacag ttttttaact agccgaacca gagaatgacc cagtggcgct  1680
gactttgctc ccgagttttt gttagaaatt accctcaaga agtaatctaa taataagggt  1740
ctctcttctg ccgtta                                     1756

<210> SEQ ID NO 104
<211> LENGTH: 1655
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 104, Example
    104: designer Anabaena PCC 7120 hox-promoter-controlled NAD-
    dependent Glyceraldehyde-3-Phosphate Dehydrogenase DNA construct
    (1655 bp)

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<400> SEQUENCE: 104

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agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc      60
gtcaagcatt tgggatgatt tccccctaca agttcctcaa attattctcc tataaacaat      120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
gaggtagata tgatggtagt taaagttggt attaacgggt tcggctcgtat cggacgtctt      240
gcattccgcc gtattcaaaa tatcgaaggt gttgaagtaa ctcgtatcaa cgaccttaca      300
gatccaaata tgcttgaca cttgttgaaa tacgatacaa ctcaaggtcg tttgatgga      360
acagttgaag ttaaagaagg tggatttgaa gtaaacggaa acttcattaa agtttctgct      420
gaacgtgatc cagaaaaacat cgactgggca actgatgggg ttgaaatcgt tcttgaagca      480
actggtttct ttgctaaaaa agaagcagct gaaaaacact tacatgctaa cggtgctaaa      540
aaagttgtta tcacagctcc tggtggaac gatgttaaaa cagttgtttt caacactaac      600
cacgacattc ttgacggtag tgaacaggtt atctcagggt cttcatgtac tacaaactgt      660
ttagctccta tggctaaagc tcttcacgat gcattcggta ttcaaaaagg tcttatgact      720
acaatccacg cttacactgg tgaccaaatt atccttgacg gaccacaccg tggtggtgac      780
cttcgtcgtg cacgcgctgg tgctgcaaat atcgttccta actcaactgg tgetgctaaa      840
gctatcggtc ttgttatccc agaacttaac ggtaaacttg acggtgctgc acaacgtgtt      900
cctgttccaa ctggatcagt aactgagttg gttgtaactc ttgacaaaaa cgtttctggt      960
gacgaaatca acgctgctat gaaagctgct tcaaacgata gcttcgggta cactgaagat     1020
ccaatcgttt cttcagatat cgtaggcgta tcatacgggt cattgtttga cgcaactcaa     1080
actaaagtaa tggaaagtga cggatcccaa ttggttaaag ttgtatcatg gtatgacaac     1140
gaaatgtctt aactgctca acttgtagct actcttaggt acttcgcaaa aattgctaaa     1200
taatgaagta agtaggaagc agggagcagg ggaaagaaaa ttgacaactg tacaagatta     1260
atcgcgtctc tgagcaatga ccaaatacat ctacctccac ggttttcttc cagcccccta     1320
tctgcgaaag cacaagatat tagcaagcgt ttgcgcaaaa ttcacataca gctaacaatc     1380
cctgatctca atgctggtag attttctcag ttaacaatca cgcgccaaat tcaacaagtt     1440
gccgcaattt tccctgataa ttctgaacca ataacgctga taggttctag tttaggcggg     1500
ttaactgctg cttatctagg acagcgatat ttacaagtac aacgcttagt tttattagcg     1560
ccagtttggt tttttatccc attggttgcc caaaatgggt gaagaagctg tcacaagttg     1620
gcaacaaacg atatagggtc tctcttctgc cgtaa                                     1655

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<210> SEQ ID NO 105

<211> LENGTH: 2303

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 105, Example
105: designer Anabaena PCC 7120 hox-promoter-controlled
Acetolactate Synthase DNA construct (2303 bp)

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<400> SEQUENCE: 105

```

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc      60
gtcaagcatt tgggatgatt tccccctaca agttcctcaa attattctcc tataaacaat      120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180

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gaggtagata	tgctaagggg	tcagctgag	ctccccaaga	cgttggttaa	agcgcagatt	240
ttcactatag	tccacagggg	catcaatgag	agtgggaaca	ttctgtgcca	aggcggtttt	300
gagggttggg	ataaagtca	tggtctctc	aatgcgatag	cctttgagtc	ccatgctttc	360
ggctaacttg	acaaaatcgg	gattactaaa	gtggacatag	gcagattcac	caaagtagcg	420
ctgctgcttc	cactcaatga	gaccatagcc	gccgtcatta	aagatgatgg	tggtaaagtt	480
tgtocccata	cgagggcgag	tttccagttc	ttggaaattc	atcatgaagc	caccatcccc	540
cgtagctgcc	accacatgcc	gctgctggata	gactaatttg	gcggccattg	cccccggtac	600
cgcaatcccc	attgcccga	agccattgga	aatcaagcag	gtgttggggc	gatcgcagtg	660
gtagtgacga	gcaatccaca	ttttatgggc	accacatca	gaaataacaa	tgtctcggg	720
ccccatgact	tgggcgaggt	catagattag	cttttggggc	ttaaccggaa	aactctcatc	780
ctgggcatat	tggtaatagt	ccgccacaat	ctcctgacgc	agttgcacgg	catagggggt	840
gggttatct	tgggcatcgg	cccgttaag	aatttcatag	agggagtcag	aaatategcc	900
gacaacctca	acgacagggg	tatagctgct	gtcaatttcc	gcaggagtgg	ccgcaatatg	960
gataatcggc	aagcggccct	cggggttcca	gctttttggg	gaatactcaa	ttaagtcata	1020
gccaacggca	atcactaagt	cagcatgatc	aaagccgcag	ctaagttaat	cccgtgttg	1080
gagccccacg	gtccacagag	caaggggggtg	ttgatagggg	atgacccctt	tgccatgaa	1140
ggtattggcc	acggggat	tcagcttttc	ggcaaatgg	gtgagggcag	cggcagcatg	1200
ggcgcgaatg	gcgccattcc	ccactaggat	caggggggtc	tcggcagcat	tgatgagttc	1260
agcggcctta	agaactctct	ggaaagaggc	ataggttttt	tcgggggagc	tgggcttaag	1320
gggagcgcce	tcggcttcca	tgggcggaat	gttttcaggc	acatcaatgt	gaacggcgcc	1380
cggcttctca	ttctgggcaa	ttttaaggc	cttgccgaca	atttctgggg	taatactagg	1440
gctggacaatc	tgggcattcc	atttggttac	ggggctaaac	atggccacca	agtccaaata	1500
ttggtgggac	tcgatgtgca	tgcgatccgt	ccccacttgc	cctgtaatcg	ccaccagggg	1560
agcgcctctg	aggttggcat	cggcaacacc	ggtcattaaa	ttcgtggccc	cggggccgag	1620
ggtagaaaga	cagaccctcg	ctttgccggt	gaggcgacca	tagacatcgg	ccataaaggc	1680
cgccccctgt	tcgtggcggg	tggttataaa	ttgaatccga	gagcagatgga	gggcatggag	1740
gacatctaga	ttctcttccc	ccggcaaac	aaaaatata	tcaacgcctt	cattttcaag	1800
gcattttacg	agtaattcgg	cggatttcat	aggatgggcg	atcgcaggca	ctgaagtaag	1860
taggaagcag	ggagcagggg	aaagaaaatt	gacaactgta	caagattaat	cgcgtctctg	1920
agcaatgacc	aaatacatct	acctccacgg	ttttcttcca	gccccctatc	tgcgaaagca	1980
caagatatta	gcaagcgttt	cgcccaaat	cacatacagc	taacaatccc	tgatctcaat	2040
gctggtgaat	tttctcagtt	aacaatcacg	cgccaaatc	aacaagttgc	cgcaattttc	2100
cctgataaatt	ctgaaccaat	aacgctgata	ggttctagtt	taggcggttt	aactgctgct	2160
tatctaggac	agcgatattt	acaagtacaa	cgcttagttt	tattagcgc	agtttggttt	2220
tttatcccat	tggttggcca	aaatgggtga	agaagctgtc	acaagttggc	aacaaacgat	2280
ataggttctc	tcttctgccc	tta				2303

<210> SEQ ID NO 106

<211> LENGTH: 1661

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 106, Example
106: designer Anabaena PCC 7120 hox-promoter-controlled Ketol-
Acid Reductoisomerase DNA construct (1661 bp)

<400> SEQUENCE: 106

```

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc      60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat      120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
gaggtagata tgtaattttt tatccttata cactatcttt ccttttccaa gccagctcat      240
catacttcta agtttagatc ccacctgctc gatgggatgg ttttcatcag ccctggtgag      300
ggcattaaat actggtctgc cagctttatt ttccagcacc cattcttttg caaattctcc      360
tgattgtatc tgtcttaaaa cttccctcat gttgtcttta acctggggat ctattaccac      420
aggccccctt gtgaggctac catattgggc tgtgttgctt atggaatata tcatgttggg      480
gataccacct tcatagagaa gatccactat cagttttacc tcatgcatac attcaaaata      540
agccatttct ggagcgtagc cagcctccac caatgtttca aaaccatatt taataagctg      600
tgtaaaacca ccacataaaa ccgctgctc accgaaaaga tctgtttctg tttcctcttt      660
gaagtttggt tcaagcacac ctgctctggc tccaccaatg gcagctcgtg atgacaaagc      720
cacctctctt gaatcaccgg aatagtcttg atgcacagcg atgaggcatg gtactccgcc      780
accctttgta tattctgctc ttacaagatg ccctggccct ttaggtgcaa tcattataac      840
gtttacgttt ttaggaggca ctatctgtcc aaaatggatg ttgaaaccgt gggcaaatgc      900
aagatatgtc ccctctttta tataaggggc aatctgctct ctgtaaagat cccctgtat      960
ctcatcagga acgagatata tgatgagtc tgccattttt gtggcctctg atatctccat      1020
aactttaagc ccggagcttt ccgctttttt ccaggaatca ccacccttc ttagggcaac      1080
ggcaacgtca acaccactat ctttgagggt attagaatgt ccataaccct gacttccgta      1140
accaactatg gccacccttt tctttttaat caattctaaa tttgcatcct tttcgtagta      1200
tactttcatt gaagtaagta ggaagcaggg agcaggggaa agaaaattga caactgtaca      1260
agattaatcg cgtctctgag caatgaccac atacatctac ctccacggtt ttcttccagc      1320
cccttatctg cgaaagcaca agatattagc aagcgtttcg cccaaattca catacagcta      1380
acaatccctg atctcaatgc tggatgaatt tctcagttaa caatcacgcg ccaaattcaa      1440
caagttgccg caattttccc tgataattct gaaccaataa cgctgatagg ttctagttaa      1500
ggcggtttta ctgctgctta tctaggacag cgatatttac aagtacaacg cttagtttta      1560
ttagcgcagc tttggttttt tatcccattg gttgcccata atgggtgaag aagctgtcac      1620
aagttggcaa caaacgatat aggttctctc ttctgccgtt a                               1661

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<210> SEQ ID NO 107

<211> LENGTH: 2324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 107, Example
107: designer Anabaena PCC 7120 hox-promoter-controlled Dihydroxy-
Acid Dehydratase DNA construct (2324 bp)

<400> SEQUENCE: 107

```

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc      60

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gtcaagcatt	tggtatgatt	tcccctcaca	agttcctcaa	attattctcc	tataaacaat	120
agatataagg	tcaaaacttg	agttatgagt	gctgagtaaa	aaattactct	ccacgcctca	180
gaggtagata	tgttataaat	ccgttatata	tccaaaagat	gctgatgata	ccagcttgct	240
atacttcctt	aagcttccac	tcacatttcg	ctcgggcttt	ataggagttt	gctcctttcg	300
tttttcccat	tctcttctct	ccatcaatac	actaattgaa	ttttccactg	catctattct	360
gatgcggtcg	ccattttcta	cataagctaa	tgcgccatta	tcaaaagctt	cggttgaaat	420
gtgtcccact	acaaaaccgt	gagttcctcc	tgagaacctt	ccatctgtaa	tgagggtac	480
tttctttccc	aaacctgctc	ccattatagc	agcagttggc	tttaacattt	cgggcattec	540
aggacctcct	tttggccctt	catatogaat	caccactaca	tcacctctt	ttatctcgcc	600
tctggcaata	ccatcattgg	ccagaaatc	accatcataa	actctagcag	ttccttcgaa	660
tatctctctt	tcctttccgg	tgatttttagc	aacagctcct	ccaggtgcta	aattgccttt	720
caatattcgc	aaatgacctg	agcttttgat	tggatttttc	acagagttaa	tgatgtcttg	780
cttttcgcct	aaacctgaa	catctttata	atcttcagct	aaagtcttac	ctgttatggt	840
catgcaatcg	ccatgaagaa	gtccttcccc	caacatcatt	ttcatcactg	caggaatccc	900
gccattttga	tgaagatctt	ccatcaagta	tttaccagaa	ggctttaagt	cggttagata	960
aggagtctcg	gcaactgatc	gagcgaaaac	ttccaatggt	aaatcgattt	caaaaagcatt	1020
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tatggcggtt	tcaaatgatt	tttttagtgac	gatatcccgt	ggcttcaaat	cattttcaat	1140
caatttttta	atggctaaac	cagcctctgc	acattcttcc	cttttctcag	cactttgagc	1200
gggattggaa	gcaccataag	gcaagcataa	tcctaaggct	tcaatagcgg	atgccatcgt	1260
attggcagtg	tacattcctc	cacaggtccc	aggacctggg	catgcatttg	ccactactcc	1320
cttaaaatct	tcttcagaaa	tagtggtggc	ttgcttctta	cctaaagctt	caaaaagcaga	1380
aaaccacatct	aatttttctc	cattatggca	acctggagca	atggttctcc	cataaattat	1440
gagtgaaggt	cgattcaatc	tgcccatagc	cagtaaggcg	cctggcatat	ttttatcgca	1500
tcccgttaata	gcaatcatgg	catcgtaagc	ttgcgcatcc	actaccgttt	ccatggaatc	1560
ggctatgata	tcgcggaag	gcaatgaata	gcgcatcccg	ttggtaccca	tagaaatacc	1620
atcgcttact	ccaatagtat	tgaaaatcaa	ccctaccac	tcataagatt	tgatgctttt	1680
cttgacctct	acagccaaat	cattcaaatg	catattacat	ggattgcctt	caaaaaccagt	1740
gcttgcaata	cctatttggt	gtttttccaa	atcttcatca	gacaaacca	tggcatgcaa	1800
catggcttga	gcggcaggtt	gtgtaggatc	ttgtgtaact	gctttactat	atggatttaa	1860
ttccttcgcc	attgaagtaa	gtaggaagca	gggagcaggg	gaaagaaaat	tgacaactgt	1920
acaagattaa	tcgctctctt	gagcaatgac	caaatacatc	tacctccacg	gttttcttcc	1980
agccccctat	ctgcgaaagc	acaagatatt	agcaagcgtt	tcgcccacaa	tcacatacag	2040
ctaacaatcc	ctgatctcaa	tgctgggtgaa	ttttctcagt	taacaatcac	gcgccaat	2100
caacaagttg	ccgcaatctt	ccctgataat	tctgaaccaa	taacgctgat	aggttctagt	2160
ttaggcggtt	taactgctgc	ttatctagga	cagcgatatt	tacaagtaca	acgcttagtt	2220
ttattagcgc	cagtttggtt	ttttatccca	ttggttgccc	aaaatgggtg	aagaagctgt	2280
cacaagttgg	caacaaacga	tataggttct	ctcttctgcc	gtta		2324

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<210> SEQ ID NO 108
<211> LENGTH: 2288
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 108, Example
108: designer Anabaena PCC 7120 hox-promoter-controlled branched-
chain alpha-Ketoacid Decarboxylase DNA construct (2288 bp)

<400> SEQUENCE: 108

agaaaatctg gcaccacacc gcagaaatag aggggctagg agttgagggt actctggttc 60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgtatac agtaggagat tacctggttag accgattaca cgagttggga 240
attgaagaaa tttttggagt tcctgggtgac tataacttac aatttttaga tcaaattatt 300
tcacgcgaag atatgaaatg gattggaaat gctaataaat taaatgcttc ttatatggct 360
gatggttatg ctgcgtactaa aaaagctgcc gcatttctca ccacatttgg agtcggcgaa 420
ttgagtgcga tcaatggact ggcaggaagt tatgccgaaa atttaccagt agtagaaatt 480
gttggttcac caactcaaaa agtacaaaa gacggaaaaat ttgtccatca tacactagca 540
gatggtgatt ttaaacactt tatgaagatg catgaacctg ttacagcagc gcggtcttta 600
ctgacagcag aaaatgccac atatgaaatt gaccgagtac tttctcaatt actaaaagaa 660
agaaaaccag tctatatata cttaccagtc gatgttgctg cagcaaaaagc agagaagcct 720
gcattatctt tagaaaaaga aagctctaca acaaatataa ctgaacaagt gattttgagt 780
aagattgaag aaagtttgaa aaatgcccaa aaaccagtag tgattgcagg acacgaagta 840
attagtttgg ttttagaaaa aacggtaact cagtttggtt cagaacaaa actaccgatt 900
acgacactaa attttggtaa aagtgtctgt gatgaatctt tgcctcatt ttttagaata 960
tataacggga aactttcaga aatcagctct aaaaattttg tggagtccgc agactttatc 1020
ctaatacttg gagtgaagct tacggactcc tcaacagggt cattcacaca tcatttagat 1080
gaaaataaaa tgatttctact aaacatagat gaaggaataa ttttcaataa agtggttagaa 1140
gattttgatt ttagagcagt ggtttctctt ttatcagaat taaaggaat agaatatgaa 1200
ggacaatata ttgataagca atatgaagaa tttattccat caagtgtctc cttatcacia 1260
gaccgtctat ggcaggcagt tgaaagtgtg actcaaagca atgaacaat cgttgctgaa 1320
caaggaacct ctttttttgg agcttcaaca attttctta aatcaaatag tctgtttatt 1380
ggacaacctt tatgggggtc tattggatag acttttccag cggctttagg aagccaaatt 1440
gctggataag agagcagaca ctttttattt attggtgatg gttcacttca acttaccgta 1500
caagaattag gactatcaat cagagaaaaa ctcaatccaa tttgttttat cataaataat 1560
gatggttata cagttgaaag agaaatccac ggacctactc aaagtataa cgacattcca 1620
atgtggaatt actcgaatt accagaaaaca tttggagcaa cagaagatcg ttagtatca 1680
aaaattgtta gaacagagaa tgaatttgtg tctgtcatga aagaagccca agcagatgct 1740
aatagaatgt attggataga actagttttg gaaaaagaag atgcgcaaaa attactgaaa 1800
aaaatgggta aattatttgc tgagcaaaaat aaatagttaa gtaagtagga agcaggggagc 1860
aggggaaaga aaattgacaa ctgtacaaga ttaatcgcgt ctctgagcaa tgaccaata 1920
catctacctc cacggttttc ttccagcccc ctatctgcga aagcacaaga tattagcaag 1980

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cgtttcgccc aaattccat acagctaaca atccctgatc tcaatgctgg tgaattttct	2040
cagttaacaa tcacgcgcca aattcaacaa gttgcccga tttccctga taattctgaa	2100
ccaataacgc tgataggttc tagtttaggc ggtttaactg ctgcttatct aggacagcga	2160
tatttacaag tacaacgctt agttttatta gcccagttt gggtttttat cccattgggt	2220
gccccaaatg ggtgaagaag ctgtcacaag ttggcaacaa acgatatagg ttctctcttc	2280
tgccgtta	2288

<210> SEQ ID NO 109

<211> LENGTH: 1613

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 109, Example
 109: designer Anabaena PCC 7120 hox-promoter-controlled
 2-Methylbutyraldehyde Reductase DNA construct (1613 bp)

<400> SEQUENCE: 109

agaaaatctg gcaccacacc gcagaaat at aggggctagg agttgagggt actctggttc	60
gtcaagcatt tgggatgatt tccccctaca agttcctcaa attattctcc tataaacaat	120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccaagcctca	180
gaggtagata tgatggcttc tgtaaatgac tactttgaga acgccaagac gacgtacttt	240
actttgagat cgggtgacaa gatccccgct gttggattgg gtacttggca atcaccaccc	300
aacgagacta aagaggcagt caagtacgct ttgcagcacg gttaccgtca catcgatgct	360
gccgccattt atggtaacga agacgaggtt ggtgacgcta tcaaggagag tggaaatccct	420
cgtgacccaa tctgggtcac atctaagctc tggtgcaatg ctcatgctcc cgaggctgtc	480
ccaaggctt tggagaagac cttgcgtgag ctgaaacttg attacctga cctttacttc	540
atccactggc ctatttcttt gaagaccggc gatgacttgg ttcccaagga caaggacggc	600
aacaccatca ctgtcgaat tccccctgag gacacctgga aggctatgga gggctctgtg	660
aagtccggca aggtgaagaa cattgggtatt tccaatttca acaacgaaga gttggatcgt	720
attttgaagg ttgccagat tccctctgcc gtccacaaa tggaaactca tccttacttg	780
aagcagacgg agttcattga gaagcacaag aagcttggca ttcacgtcac cgttactctg	840
cctttggcca accaaaatgc tctttacggc aatgccgttc ccaagttgat tgagcacaag	900
actcttctgc acattgccc gaccagggtt gagggcgtca ctggtgccc cattgctatt	960
tcttgggcag tcaagcgcgg tacttccggtt attcctaagt ctgttcatgc caacagaatt	1020
aagagcaact tcctcgttgt tcccttgact gatgacgaga tgaaggccat cgataacatt	1080
ggtgtcagca agcgtttcaa ttggagcaaa gttttctgca atgagaattg tttctacggt	1140
cttgaggatg gtccctcagta atgaagtaag taggaagcag ggagcagggg aaagaaaatt	1200
gacaactgta caagattaat cgcgtctctg agcaatgacc aaatacatct acctccacgg	1260
ttttcttcca gcccctatc tgcgaaagca caagatatta gcaagcgttt cgcccaaat	1320
cacatacagc taacaatccc tgatctcaat gctggtgaat tttctcagtt aacaatcacg	1380
cgccaaatc aacaagtgc cgcaatttcc cctgataatt ctgaaccaat aacgctgata	1440
ggttctagtt taggcggtt aactgctgct tatctaggac agcgatattt acaagtacaa	1500
cgcttagttt tattagcgc agtttggtt tttatcccat tggttgccc aaatgggtga	1560
agaagctgtc acaagttggc acaaacgat ataggttctc tcttctgccc tta	1613

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<210> SEQ ID NO 110
<211> LENGTH: 1300
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 110, Example 110: designer Prochlorococcus marinus MIT9313 groE-promoter-controlled NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase DNA construct (1300 bp)

<400> SEQUENCE: 110

```
agaaaatctg gcaccacacc ccctttcaga gcggcgcaac attaccactg catggcgaga    60
tcttctcagg gttcggtgac cgcacacagt atccactagt cggcacagca tcaacacaca    120
tagggttggc actcaatggc cacgagtgct actcatgtta tgccaagccg actttacgaa    180
ccaattccgc ggtgocgctg gcgtaacca tttcgttgtc ataccaagtg tagattttca    240
ccattcgctt accaaccacc atggtggaga ggcacccac aattggtgaa cgttgatcgc    300
cttggaatc aatcgacacc agtggacgct cttcaaaacc cagaatgcct ttaagctcgt    360
tttctgagge ttgttttaac agctgattga tctcttcac cgtcgtatca cgtgcacat    420
caaaaatgat gtcggttaac gaggcattcg ccaacggtac acgtacggcg tgtccatcaa    480
tcttgccctt cagatccggg aaaatttcga taatcgcttt agcagagccg gtggttgagg    540
ggatgaggct cataccgcaa gcacgtgac ggcgtaaatc tttatgaggc gcacccaaaa    600
tggtttgctg attggttagg ttatggatgg tagtaaaaga ggcttgtgcg ataccagtt    660
ttcatggat tactttcacc actggagcaa tacagttagt ggtacaagaa gcggcagtga    720
caatgcgatg ttgctccgga ttgaagatgt gatcgttcac cccgaccacg atattggcga    780
tcccctcttc tttgacagge gctgaaacga cgacgcgctt tactccttgt gccaaatact    840
ggttcaagaa ttcgccttta cggtgcttac ccgtagcctc aatcaccaca tcacagcccg    900
accaatccac tgcataatc gatttttctt gtggtgtgcy aatgcgcttg ccggtgatca    960
ggatagcatc cgcttactg cccacagcat gatgccaacg accttgtacc gaatcgaact   1020
ccaaaaggty cgtaaatgtt gcagcatcac ccgccacatc gttgatctgt acaaaactca   1080
tctcaggcca atcaaacgaa gctcttaaag ccaaacgccc aatcagacca aatccattaa   1140
ttccgacttt aattgccatt gatttagttt cgggtgtctat ctcttaatag cctcgattta   1200
ttttcggggc tattaatcaa ctctcagagg cgacaagctt cttcttcctt tacgacgctt   1260
ttattgggtg gacatggcaa ggttctctct tctgcccgtta
```

<210> SEQ ID NO 111
<211> LENGTH: 1498
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 111, Example 111: designer Prochlorococcus marinus MIT9313 groE-promoter-controlled Phosphoglycerate Mutase DNA construct (1498 bp)

<400> SEQUENCE: 111

```
agaaaatctg gcaccacacc ccctttcaga gcggcgcaac attaccactg catggcgaga    60
tcttctcagg gttcggtgac cgcacacagt atccactagt cggcacagca tcaacacaca    120
tagggttggc actcaatggc cacgagtgct actcatgaaa tatgtaatct tacttggcga    180
cggcatggcc gacgaaaaaa ttgccgaact ggacggcaaa acgctcttc aatcgcgag    240
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cacaccaat atggacaggc tggcggcagg cggggaaacc ggcattgtac ataccgtccc 300
ggatggattt ccgcctggca gcgatgtagc caacctctcg gtaatggggt acaatccgcg 360
ggaatattat accgggctgt ctccgctgga agcggtaagc atgggggttg aactttcaga 420
tgacgatgta gccttccgct gcaacctggt taccctgtca gaagaagagg tatatgaaaa 480
caagattatg gtggattaca gctcggacga aattaccacc tctgaatcgc atgaactgat 540
cagggaaagtc gccaacccgc tgggcagtaa agagttgcgc ttttaccocg gtttcggatt 600
tagacacctt cttgtatgga aaacagggcc tgttggcggg aagctaacac caccccacga 660
tatctcgggg cgtaccattg ccccatacct ccccaaaggc gagggtagcg acactttaa 720
gcggttaatg aaagaaagca acaggttctt gccagagcac ccggttaacc aaaaaagggt 780
aaggcccggt cttaggcctg ccaacttccat ctggttcttg ggacagggaa agaaacctc 840
gataccgaag ttctacgaca aatacggggt aaccggctct gttatctctg ccgtggacct 900
gattaagggg attggcatct gtgccgctt cgatatagtt aaggtggagg gtgtaacggg 960
caccatccat accaacttcc gggggaaagt gcaggccgcc ctggaagaac tgaaaaaggg 1020
aaaggacctg gtctacatc acggttaggc tccggacgca gcaagccaca ggggtgaaac 1080
tgttacaaa gttaaagcta ttgaaatggt ggacaacatg ctgggccagc ttttaaaaa 1140
actggacgaa ttcggcatgt acaaaataat gctcttgccc gatcatcaa ccccgctcag 1200
cactaaaacc cactctaaca gccctgtccc cttgttatc tatccaagg ggcggaaaaa 1260
taaaagcgcc gcttcttttg acgaagaaac ggcggcaaaa agcggacttg tttccgggc 1320
ggccatgag ttaatggatt actttatccg cagctaatga tttagtttcg gtgtctatct 1380
cttaatagcc tcgatttatt ttcggggcta ttaatcaact ctcagaggcg acaagcttct 1440
tcttcctta cgacgtttt attggttga catggcaagg ttctctcttc tgcggtta 1498

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<210> SEQ ID NO 112

<211> LENGTH: 1588

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 112, Example
112: designer Prochlorococcus marinus MIT9313 groE-promoter-
controlled Enolase DNA construct (1588 bp)

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<400> SEQUENCE: 112

```

agaaaatctg gcaccacacc ccctttcaga gcggcgcaac attaccactg catggcgaga 60
tcttctcagg gttcggtgac ccgcacaggt atccactagt cggcacagca tcaacacaca 120
tagggttggc actcaatggc cacgagtgct actcatggtg tacgtggaaa tcgtggatgt 180
aagagcaaga gaggtcctgg attcgagagg aatcccacc gttgaagcgg aagtcgtgct 240
tgaagacgga acaatgggaa gagccatcgt gccctctggt gcctccactg gaaaattcga 300
agccctggaa atcagagaca aagacaagaa gagatactc gggaaaggtg tctgaaggc 360
cgtagagaac gtgaacgaaa ccatagctcc cgcgctgatt ggaatgaacg cattcgacca 420
gccactcgtt gacaagacac tgatagaact ggatggcaca gagaacaaat ctaaactggg 480
tgccaacgct atactcgccg tttctatggc agttgccaga gcggcggcga attacctcgg 540
attgcccctc tacaataacc ttggaggagt caacgcaaag gttctgccag tacctttgat 600
gaacgtgatc aacgggtggac agcacgcaga caacaatctt gacctcagg aattcatgat 660

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cgttcccgcc ggatttgaca gcttcagaga agctttgagg gcaggagcgg aaatattcca 720
cacgttgaaa aagatactcc acgaagccgg tcacgtgaca gcagtaggag acgaggggtg 780
attegacccc aatctgtctt ccaacgaaga agccataaag gttctgattg aagccataga 840
gaaagctggc tacaagcccg gagaagaagt cttcatagct cttgattgcg cagcatcttc 900
cttctacgat gaggaaaagg gagtttacta cgtcgtatggt gaagaaaaat ccagcgaagt 960
tctcatggga tactacaag aactgggtggc gaagtacccc atcatatcca tcgaagatcc 1020
gttcgcggag gaagactggg atgcatttgt ggaattcaca aagagagtag gaaacaaggt 1080
tcagatcgtt ggagatgacc tttacgtgac caacgtgaaa agactttcca aaggaataga 1140
actcaaagcg accaactcca tactcatcaa actcaatcag ataggcaccg tcacggaaac 1200
tctcgcgcg gtggagatgg cacagaagaa caacatgaca gccatcattt cccacagatc 1260
tggagagagt gaagacacgt tcattgcgga tctcgtctgt gcaacgaacg ctggtttcat 1320
caagacaggt tcctctcca gaagcgaag gatagccaag tacaaccagc ttttgagaat 1380
cgaggaagaa ctcgaaaaag tggcagaatt cagaggtttg aaatctttct actctataaa 1440
gagataatga tttagtttcg gtgtctatct cttaatagcc tcgatttatt ttcggggcta 1500
ttaatcaact ctcagaggcg acaagcttct tcttccctta cgacgttttt attggttggga 1560
catggcaagg ttctctcttc tgccgtta 1588

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<210> SEQ ID NO 113

<211> LENGTH: 1717

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 113, Example 113: designer Prochlorococcus marinus MIT9313 groE-promoter-controlled Pyruvate Kinase DNA construct (1717 bp)

<400> SEQUENCE: 113

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agaaaatctg gcaccacacc cctttcaga gggcgcaac attaccactg catggcgaga 60
tcttctcagg gttcggtgac cgcacaggt atccactagt cggcacagca tcaacacaca 120
tagggttggc actcaatggc cacgagtgt actcatgatg agaaaaacca agcttatatg 180
ttcgatagga cctaaaaccg agaaaccaga gaaaataaag gaattgctaa aaagaggggt 240
aaatgctttt aggataagcg cagttcatta cacgatggaa aaaatcacag agctggtaga 300
gctaatacaa gatattagat atgaactcaa aatgcctggt tccatcattc ttgatttacc 360
aggttgcaaa ctccggacgg gagatcagaa agaagaaatc atcgaactta gacaagggtga 420
aaaagtgact gtaacaagcg aaaaaacttt ttcttctcgg gatactataa gcataaattt 480
ttccggacca tttcaggggtg taaaaaccgg cgatttaatc ctcgtagatg atggaaaaat 540
acaactcaga gtggaagaaa tttctccaaa aaaagtagaa tgcgtcgtgg agataggagg 600
aattttgaag aaaaacagtg gcgtcaattt tcccaattct gatctactg tcgaagtacc 660
aacagaagaa gatattaaga tcatagctga aactgtcaat atgggactgg actattactg 720
tgtatcattt gcaagaaacg caaaagatgt tcaaaaaatc aaaaaacatc ttgaatcttt 780
cgattcaagt gcgaaaaatc ttacgaaaat agaacaacaa aaatccatag aaacgctgga 840
agatatatgt cgcgtgagcg atggaataat cgtagcaaga ggagatttag cggtgagac 900
atctctgata gatttgccga tattgcaaaa aaagataata aataccgcat caaattataa 960
aatacctgtg atcgtcgcga ccgaaatact caattctatg atcaacagtt catcaccaac 1020

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aagggttgaa ataatggatg ttgcaaacat agtttttagat ggagcagatg ctatactgct 1080
aacctctgaa acagctgtgg ggaactttcc gatcgaaaca gttgaaaaaa ttaatgagat 1140
tgttgaaaat gttgagaact acctaccgga aatcaatgct ctttttaaag aacgcagggt 1200
tgaaaaaatc gaagatccat ctgaagctat tgcaaggagt agttactaca tttctgaaga 1260
aataaatgcc aaagctataa taatatcaac agcttctgga agcactgcaa gaagggtggc 1320
ctatttcaag ccacttcgtc ctatcatagc tacgacccca gatgaaaaaca cttttcatca 1380
gttgtctatt gtttggggga tagttccgat gctaattcca gaagtccatt ccacagatat 1440
aatgatccac gtggccgctcg agaaggtaa agctgtcggga tatgttcaaa attccgacat 1500
tgtggttgtt acttctgttg ctccgtgtgg tattgttggga acaactaaca tgctcaaagt 1560
tcacatagtt gagtagtgat ttagtttcgg tgtctatctc ttaatagcct cgatttattt 1620
tcggggctat taatcaacte tcagaggcga caagcttctt cttcccttac gacgttttta 1680
ttggttggaac atggcaaggt tctctcttct gccgta 1717

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<210> SEQ ID NO 114

<211> LENGTH: 2017

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 114, Example
114: designer Prochlorococcus marinus MIT9313 groE-promoter-
controlled Acetolactate Synthase DNA construct (2017 bp)

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<400> SEQUENCE: 114

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agaaaatctg gcaccacacc ccctttcaga gggcgcaac attaccactg catggcgaga 60
tcttctcagg gttecggtgac ccgcacaggt atccactagt cggcacagca tcaacacaca 120
tagggttggc actcaatggc cacgagtgct actcatgtca agattgetta gaggcttctt 180
tattaaaatg ttctcaaat ttttttgaa aacgctgac gcccaaatga atgttgtcgc 240
tgtaatcgac cggaatatca atgacaacag gccctctgc atcaagacca gctttaagca 300
cctctgcca ttcgtcaggt gaattgacc ttaaaccttt tgcaccaaag ctttcagcat 360
attttacaat atcgattccg ccgaagtcca ctccggacgt ccgcttgat tcatctcct 420
gctggaacgc aaccatateg tatgtgctgt cattccagac aatgtgaacg atcggcgctt 480
ttaatctgac cgctgtctca agctccatcg cggagaacag gaagcccccg tccccgaaa 540
cagacacgac tttctgtccc ggattgacca gcgttgctgc aatcgcccac ggcaaagcca 600
ccccaaagct ctgcatgccg ttggaatca gcagtccatg cggacgtag gtgcgaaat 660
atctagacat ccaaatcgca tgggagccga tgtcgcaagt caccgttatg tcatcgctca 720
gcagttcacg caaatcgca acgatttgca gcggatgaac aagatcagtt tttgttctt 780
taggagggtc gctttgctcc tccagtgtt tctcaagta atcaaggaca ggtgcaaagg 840
actcgtcgat ggaaaccggc agagaatcat gttcaatag gtttaacgtc tctcggatat 900
cgccgatcaa ctcgatttcc ggctgatagt catgatcgat atcggcttgt atttcgtcaa 960
gatgaatcac gcttcgttcc cttttccat tccaaaagac cggatcgtat tcaatcggat 1020
catagccgac cgtcaaaacg acatccgctt tttccaatag catgtctccg ggctgattgc 1080
ggaatagtc gatccggccc aagtaactgg cttccaaatc gtgagacagc gtaccggctg 1140
cttggatagt ttcaacaaac gccagtttca ctttccttag cagacgccga accgcttcaa 1200

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tcgcttcagg tcttccgcct ttcaccccga caagcacgac aggaagggtc gcattgtgaa	1260
ttttggcgat ggccgcgctg atttgttcgt ccgaagccgc gccacagcttc ggcgcccggca	1320
tggttttcac cggtttggca gttgcccggac cggccgtaac gtctctgcgga aagctgagaa	1380
acgctgcgcc agcctgtcca gaagccgccg ctctgaatgc attggttaca gcctcaggta	1440
tgttgttcgc atcttccact tctgcgctat atttcgtaat cggetgaaac aacgccgcat	1500
tatccatcga ttgatgagtt tttttgagac gatccgctct ttttacagca cccgccaggg	1560
caacaaccgg atctccttct gtattggctg ttacaagacc ggctcgctaaa ttagacgctc	1620
ccggacctga agtcaccagg caaacaccgg gctttccagt caatcgtccg actgccgccc	1680
ccataaatgc tgcattctgc tcgtgacggc aaacgatcaa ttcaggcccc ttgtctttca	1740
atacgtcaaa caccgcatcg attttcgctc ccggaatacc gaaaacatga gtgacacctt	1800
gctgaatgag actatccacc acaagctctg ctctctctac agtaagagtt tcatttttag	1860
cggtacatt attcaatgat ttagtctcgg tgtctatctc ttaatagcct cgatttattt	1920
tcggggctat taatcaactc tcagaggcga caagcttctt ctcccttac gacgttttta	1980
ttggttggaac atggcaaggt tctctcttct gccgta	2017

<210> SEQ ID NO 115

<211> LENGTH: 1588

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 115, Example 115: designer Prochlorococcus marinus MIT9313 groE-promoter-controlled Ketol-Acid Reductoisomerase DNA construct (1588 bp)

<400> SEQUENCE: 115

agaaaatctg gcaccacacc cctttcaga gggcgcaac attaccactg catggcgaga	60
tcttctcagg gtteggtagc ccgcacaggt atccactagt cggcacagca tcaacacaca	120
tagggttgga actcaatggc caccgagtgt actcatgtca ctctcatcc acgttcctct	180
ccttgagcca aggcacatt tttctgagtt cttttcctac tttttctatg agatgctctg	240
attctttctt tctcatgggt tagaagtagg gtcttcccgc ctgattttcg agtatccagt	300
ccttgccgaa ctttcccgtc tgaatgtcct tgagcatctg ctctcatgtc tccctcactt	360
cttttgtaac gatcttttcc tgactgatgt agtcaccgta ctcccggtg ttgctgacgg	420
agtatctcat gaaagagaga ccaccctcgt agatgaggtc aacgatgagc ttgagctcat	480
tgagacactc aaagtaagct atttccggtt gataaccgcc ctccacaaga gtttcgaaac	540
cagcttttat gagagccgtt actccaccac agaggaccgc ctgctctcca aacaaatccg	600
tttccgtctc ttccttgaag gtcgtctcta tcacaccgcc ccttgtcaca ccgataacct	660
tggcataaag gagcgtata tctttggctt taccgggtga gtctgatag accgctacga	720
gagccggcac accccttctc tcgacgtatt ctcttctcac gatgtgacca ggcctcttcg	780
gagcgcacat cgtcacatcc acgttcttcg gaggtatgat ctggtgatag tggatgttga	840
accctggggc gaacatcagc atcttaccct cggtgaggtg tttttctatg tattttttgt	900
agatctccgg ctggttctca tctgggatga gcatcatgat gatgtcggcc tcttttgccg	960
cttctctcat tgccttcaeg gtgagacctt gttctcctgc cttcttcag ctcttgcctc	1020
cctctctcaa tccgaccaca acggtgagac cgctgtcttt cagattcaac gcgtgcgcat	1080
gccctgact tccgtaccct atgatgcga tctttttgtc cctgatcagt tcgagatccg	1140

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cgtctttgtc ataataaatc actgccattg atttagtttc ggtgtctatc tcttaatage 1200
ctcgatttat tttcggggct attaatcaac tctcagaggc gacaagcttc ttcttccctt 1260
acgacgtttt tattggttgg acatggcaaa acaatccagt cgagagctag cgttgaacg 1320
ccgtaaggcc ctgagtaatt caggaagaa atcaaccaca ttaatggat caagtccctaa 1380
tcgcatccgt actgcctctg atgcacgtct aaccaggact gatcaatctt tcgttaaggc 1440
tgggaaagaa tctgtgcagc taaccgctcc taagagagag caactagata cgtctttgt 1500
tgcttctaga gaatcatccg gagcttcgcg cgtcaagtg aaaacgatcc gaaattcaag 1560
cagagaatgg ttctctcttc tgccgtta 1588

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<210> SEQ ID NO 116

<211> LENGTH: 1960

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 116, Example
116: designer Prochlorococcus marinus MIT9313 groE-promoter-
controlled Dihydroxy-Acid Dehydratase DNA construct (1960 bp)

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<400> SEQUENCE: 116

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agaaaatctg gcaccacacc ccctttcaga ggggcaaac attaccactg catggcgaga 60
tcttctcagg gttcgggtgac ccgcacaggt atccactagt cggcacagca tcaacacaca 120
tagggttggc actcaatggc cacgagtgtc actcatgcta atctttcaag atagcgctg 180
tactggcgga ttgcaccatt tttgaatata ttttcatata gcctgagttt atcttgggtt 240
ccggaactcg ccaactgcgtt ctccctggcag caagtccctc gtcgctaagt cgtacactaa 300
ggctgtgggt cggatatatca atgacgatta tgtctccgct ctgaataaga gcaatatgtc 360
ccccctgtgc cgctcgggga gatacgtgcc ctatagaagc tccccgggta gcaccagaaa 420
aacggccatc ggttataaag gctacgtcct tgtctagccc catccccgct atagcagaag 480
taggcgtgag catttccctc atccctggcc ctccgcgagg cccttcatac cggattacga 540
cgacttctcc tttttcaatc ttgcctccta gaatggctgc aacagcttct tcttccgagt 600
cgaaaacccc tgctttaccg tcgtgataga gcattgctgg gtcactgccc cccttcttca 660
ccaccgcgcc ctccctggcc aggttaccga acagtatagc caaccccccg gtagtactgt 720
gcggtattctc tatactacgg atgacctcgt ggtccttcac agggtagtca ttgataacct 780
ccccaacctg cttaccagta accgtcaggc actggcggtt taccagccct gccttgtcta 840
actcgttgaa aatggcctga actccaccg cagcgtacaa gtcttctata aaatggttgc 900
cggcagggtc gattttacat aggtgaggag tggatcgcct gatatggtt atcaggttca 960
agtccagctt aacgcccggc tcgtgagcga tagccataag atgcaaaccc gtattggtag 1020
aacaacctat tgccatgtcc aggcgtaagg cattgatgaa agcctcttgg gtcgatgat 1080
ccccgggctt tatgtccctt tcccatagct ccatcacttt catgcccgcc tgtttggcta 1140
gacggattcg ttcagagtgt accgcgggta tagttccggt cccgggtaag cccattctca 1200
ccgcttccgt caggcagttc atggaattag cggtaaacat accggcacag ctgccacaac 1260
ccgggcaagc tacatcttca agctccgcta agtccgaaag cgacatctta cctgcgctga 1320
ccgcgcctac cccttcaaag accgtggtga ggctgacctt gcgccccggg aaattgccag 1380
ccaacatcgg ccctccgctg acaaacatca aaggaagatt gaggcgagcg gccgccatca 1440

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gcatcccagg gatgattttg tcgcagttag gtataaaaac cagggcatcg aaaggatgag 1500
ccatagccat tatctcaata gaatcggcaa tcagttcccg gctggccaga gagtatttca 1560
taccgatatg gttcatggca ataccatcgc ataccctat agtagaaaac tctataggag 1620
tgcccccttt catcctcacc ccggctttga ccgcctcggc tatacggctc agatgaatat 1680
gccctggaat aatctcgttg gccgagttga ctataccgac taagggtctc tccaactcct 1740
catcgggtcaa tcctaaagct ttaaacagtg aacggtgagg tgctttttct agaccctttt 1800
tcgcagggtc acttctcatt gatttagttt cggtgtctat ctcttaatag cctcgattta 1860
ttttcggggc tattaatcaa ctctcagagg cgacaagctt cttcttcctt tacgacgttt 1920
ttattggttg gacatggcaa ggttctctct tctgccgtta 1960

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<210> SEQ ID NO 117

<211> LENGTH: 1945

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 117, Example
117: designer Prochlorococcus marinus MIT9313 groE-promoter-
controlled 2-Keto Acid Decarboxylase DNA construct (1945 bp)

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<400> SEQUENCE: 117

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agaaaatctg gcaccacacc ccctttcaga gcggcgcaac attaccactg catggcgaga 60
tcttctcagg gttcggtgac ccgcacaggt atccactagt cggcacagca tcaacacaca 120
tagggttggc actcaatggc cacgagtgct actcatgtta tgatttattt tgttcagcaa 180
atagtttacc catttttttc agtacttttg gtgcatcttc ttttgccaaa actaactcaa 240
tccagtacat tctatttgga tctgcttgag cttctttcat gacagacaca aattcatttt 300
cagttctaac gattttcgag actactcgtt cttctgttgc tccaatgat tctggttaatt 360
ttgagtaatt ccacattgga atatcattgt agctttgatt tggtecatga atttctcttt 420
cgactgtata accatcatta ttgataataa agcaaattgg attaattttt tctctgattg 480
ctaactcctaa ttcttgcaact gtaagttgaa gtgaaccatc accaataaat aaaagggtgc 540
tgctttcttt atctgcaatt tggcttccta atgctgctgg gaatgtatat ccaattgatc 600
cccataaggg ttgaccaata aatgactctt ttggttttta gaaaattgat gaagcgccaa 660
agaatgatgt ccctgtttca gcaacgattg tttcattgct ttgagttagg tttcaactg 720
cttgccatag gcggctcttg gataaaaagc catttgatgg aacaaagtct tcttgctttt 780
tatcgatata ttttcttttg tattctattc cgcttaggtc taagagagag gagatgaggg 840
attcaaaatc aaaatttttg atgctttcgt taaatatttt tccttcgtct atgttcagtg 900
aaatcatttt attttctatt aatgatggg taaatgctcc tgttgaagag tctgtgagtt 960
taactccaag catcaggatg aagtcggctg attccacgaa ttctttaaga ttaggctctg 1020
agagtttacc attatagatt cctaaaaatg aaggagagat tcatcaact gaactttttc 1080
caaaagttaa tgcgtaata gggagttttg tctttgaaat aaattgagtg actgtatttt 1140
ctaagccaaa gctaattatt tcatgtcctg taatcacgat tggttttttg gcatttttca 1200
agctttcttg aattttatc aaaatctctt ggtcacttgt atttgaagtt ggattttctt 1260
ttttcaaagg gagtgagggg ttctctgctt ttgcagcagc aacatcaact ggtaagttga 1320
tatagacagg ttttctttct tttagtagtg cagaaagtac tcggtcaatt tcaacggttg 1380
cattttctgc tgtcagtaaa gttcagagctg ctgtaacagg ttcgtgcatt ttcataaagt 1440

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gtttaaatac accgtcagcc agcgtatgat gaacaaatth tccttcattt tggacttttg 1500
atgtaggtga tcccactatt tctactactg gtaaatthtc ggcgtaactt cctgctaate 1560
cattaactgc actcaattca cctactccaa aggttgtaag aaatgcggca gctthtttag 1620
tacgagcata gccatcagcc atataagaag catttaattc attagcattt ccgaccatt 1680
tcatatcctt gcgggaaata atthgatcta aaaatthgta gttatagtct ccagggactc 1740
caaaaatthc thcaattcct aactcgtgta atcgggtctaa taggtaatct cctactgtat 1800
acattgattt agthtcggtg tctatctctt aatagcctcg atthattthc ggggtatta 1860
atcaactctc agaggcgaca agcttctctt tcccttacga cgtthtttatt ggttgacat 1920
ggcaagggtc tctcttctgc cgtha 1945

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<210> SEQ ID NO 118
<211> LENGTH: 1138
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 118, Example
118: designer Prochlorococcus marinus MIT9313 nirA-promoter-
controlled Alcohol Dehydrogenase DNA construct (1138 bp)

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<400> SEQUENCE: 118
agaaatctg gcaccacacc cctctagttg ccaacatcag ccgggttcag aatgtacaca 60
aagacaccaa thcttgaatt tcacacaaat gctcagthtt gttcaatctg ataccgcaa 120
taccctcttc catcaggctt aatgactcca thcgcaactt acccttgctt caataatggc 180
agggcctaaa aacctaggct atcgaatcgc cgaataaaat ttaacaaca tgatcacttc 240
thttcaagtt taattcaac aaaatthcat gttgtcaccg gtggagcga tgggatcggc 300
aaggcgatcg ctagagcaat tcgcaaaaca gggagcgaac gtcgtgatca tcgaccgca 360
tattcaaaac ggtgaagcgt tcgccgcgca attgcaatcg gacgggttcg aggcgatctt 420
tgtggcggcg gatgtcggga aggtggacga tattgaacgg thttgacaag aagctgcccg 480
ccgcttcggc cgcattgact atthgatcaa caatgctggc gtctcacgct ggaagtcgcc 540
gtatgagctg acggttgagg agtgggatga cgtgctgtca acgaatthgc gcagcgttht 600
thttgcttct cgagaagcag ctaaatatat gcgccgcaat gcaaaaggcg gagcaatcgt 660
caacattgcc tcgacaaggg cgctcatgtc cgagccgaat tccgaggcgt acgctgcatc 720
gaaaggcggc cttgtcgtt tgacctatgc gctggcggtg tcgtthtcgg atgatcgcac 780
tcgctcaat tgcacagcc ccggttgat tgaaacgggc gattatgggc aactgcgaga 840
cattgaccac cggcagcacc ccggccggcg cgtcggcaaa ccggatgata tcgcccgcc 900
thgtctgtat thatgcgat aggaaaacga thttatcacc ggggtaaat thgtcatcga 960
ccggggaatg accaggaaaa tgatttatat tgagtatga thtagthtcg gtgtctatct 1020
cttaatagcc tcgatttatt thcggggcta thaatcaact ctcagaggcg acaagcttct 1080
tcttccctta cgacgthttt atthggttga catggcaagg thctctcttc tgcctgta 1138

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<210> SEQ ID NO 119
<211> LENGTH: 1816
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 119, Example
119: designer Prochlorococcus marinus MIT9313 groE-promoter-

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controlled 2-Isopropylmalate Synthase DNA construct (1816 bp)

<400> SEQUENCE: 119

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agaaaatctg gcaccacacc ccctttcaga gggcgcaac attaccactg catggcgaga    60
tcttctcagg gttcggtgac cgcacaggt atccactagt cggcacagca tcaacacaca   120
tagggttggc actcaatggc cacgagtgtc actcatggtg agccagcgcg tttatatattt   180
tgacaccact ttgaggggac gcgagcagtc gcccgcgta agcctgaacg taggggagaa   240
ggtgcaaatt gccaggcagt tagccaagct cggggtggac ataattgagg cggcctttcc   300
gattacctcg ccgggggact ttaaagccgt aagcgaaatt gcccggcagg tgaaggcgt    360
tacggtggcc gccttgcca gggccaactt ccaggatata gaccgggctc gggaggccgt    420
gcgccacgcc gagcagccgc ggattcatac ctttattgcc acttccgaca ttcatttaaa   480
atacaagctg cgcacagacc gggaggaagt cctggatgcg gcggtggcgg cggtaaagcg   540
cgccagggcc tacaccggcg atgtggagtt ttcggcgag gacgcctccc gctccgacct   600
ggacttcctc tgccgggtgc tggccgcgpc cattgaggcg ggggctaccg taataaatat   660
accggatacg gtcggttatg ccgttctcga ggaatggggg aaatttatca atactattta   720
tcataaagt ccggaattg aaaaggtcat tgtcagcgtg cactgccaca acgacctggg    780
catggccgctg gccaaactccc ttgctgccgt aatgaacggc gccaggcagg tggaaaggggc   840
catcaacggc attggcgagc gggcgggaaa cgctgccatc gaagagatgg taatggccct   900
ttataccgct aaagatcagt acaaccttta caccaacatc aaaaccgagg aaatttacag   960
gaccagcaag ctggtgagcg ccctgacggg catgaaggtg cagccgaaca aggccgtggt  1020
gggcaaaaac gcctttgcc cagagcccg cttcaccag gacggggtgc tgaaggagcg   1080
caccacctac gagataatga acccgccat ggtagggatc agcaagagca acctggtgct   1140
gggcaagcat tccggcgcg atgcattccg ccaccggctg gaggaaatgg gctacaatct   1200
ttcggaacga gagctgaaca gcgcctttga gcgcttcaa aagctggcgc acaagaagat   1260
ggagattacc gacgaagacc tggaaagccat tatagaagaa gaaatgcgcc ttgtgccgca   1320
cacctacacc cttgagtacc tgcattttc cagcggcacc acggtggtgc ctaccgccac   1380
ggtgggctta aagcgggagc ggcagcttat ggaagaggcg gcctgcggca acggcccggg   1440
ggacgccatc tgcaaggcaa ttgataaaa aacggggctt aactgcacca tgacgagctg   1500
gggaatcaac gccgtcactg cgggcaagga cgcccttggc gacgtcagcc tgaaggtgac   1560
cgccgacggc gagaagggtt acggtgggcg cggaatcagc accgatgtgc tggaggccag   1620
cgccaaagct tacgtcaacg cggtaacaa actcatctgg gattcgcaga aataatgatt   1680
tagtttcggt gtctatctct taatagcctc gatttatatt cggggctatt aatcaactct   1740
cagaggcgac aagcttcttc ttcccttacg acgtttttat tggttggaca tggcaagggt   1800
ctctctctg cegtta                                     1816

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<210> SEQ ID NO 120
<211> LENGTH: 2199
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 120, Example
120: designer Prochlorococcus marinus MIT9313 groE-promoter-
controlled 3-Isopropylmalate Dehydratase DNA construct (2199 bp)

<400> SEQUENCE: 120

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tagggttggc actcaatggc cacgagtgt actcatgatg gccatgacca taaccgaaaa 180
aattctggcc gatcacgccc gcaaaaagca ggttgagccc ggcgaactga tcagcgtaaa 240
ggttgatctg gtgctgggca acgacataac ggcgcccgtg gcgattaaag agtttgagaa 300
aataggggtg gcggaagtct ttgaccggga gcgggtggcc ctggtcccgg atcactttac 360
ccctaacaag gacattaagt cggcgggaaca gtctaaaatt ctaagggagt tttccaaaaa 420
gcacaacctt gccaaactatt tcgaggtggg cgggcccggc attgagcact gccttctgcc 480
cgaggaaggc ctggtaggccc cggcgacct ggttatcggc gccgactcgc acacctgcac 540
ctacggcgcc ctgggggccc tctccacggg cgtgggcagc accgacctgg cggctgccat 600
ggcgctgggg gaaacctggc tgaagtgcgc ggagtcaatc aaattcgaat atgacgggga 660
aatgcagccc tgggtaggcg gcaaggacat gatcctgcac acaatcgggg atatcggggg 720
ggacggggcc ctttacaagg ctatggagtt taccggcccgc gccgttgaat aactttccat 780
ggacggggcc tttaccatgt gcaacatggc cgtagaggcc gggggtaaga acggcattat 840
tgctccggac gaaacaaccc gggctctatg cggggccgc tgcaagcgc cctatcgttt 900
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caacattgaa atcgatcagg ttgttatcgg ctcctgcacc aacggccgga tggaggacct 1080
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tgtcgaagct ggccggagtgc tgagcagccc cacctgcggg ccctgcctgg gcggccactc 1260
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cgtgctgggc cggataggcg gtccatggga ggtggattga ccctttcaga gcggcgcaac 1440
attaccactg catggcgaga tcttctcagg gttcgggtgac cgcacaggt atccactagt 1500
cggcacagca tcaacacaca tagggttggc actcaatggc cacgagtgt actcatgatg 1560
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gaccctcct tccccccc ggtcaggccc ggcgacgtga ttgtggccgg caagaatttc 1740
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attttcgagt ctccccgaag cgcggggggc attggccagg gcgacgaggt ggcgggtggc 1920
gcggctgccc gcattataac cgacctgacc accggcaaga cctaccgggc ggcgcccgtt 1980
ccgccttca tcggcgagat cattgcccgc ggagggctga tcaattacgt ggcggggaag 2040
gtgagaggca atgcataatg atttagtttc ggtgtctatc tcttaatagc ctcgatttat 2100
tttcggggct attaatcaac tctcagagcc gacaagcttc ttcttccctt acgacgtttt 2160
tattggttg acatggcaag gttctctctt ctgcccgtta 2199

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<211> LENGTH: 1378
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 121, Example
    121: designer Prochlorococcus marinus MIT9313 groE-promoter-
    controlled 3-Isopropylmalate Dehydrogenase DNA construct (1378 bp)

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<400> SEQUENCE: 121
agaaaatctg gcaccacacc ccctttcaga gggcgcaac attaccactg catggcgaga    60
tcttctcagg gttcgggtgac cgcacacaggt atccactagt cggcacagca tcaacacaca    120
tagggttggc actcaatggc cacgagtgct actcatgcta cacctcgacc tectcaactt    180
ttctcgctac taaatcacc atttccttgg tgtaaccag cttctgatcc ggctccgtaa    240
tgtccggcgt ccggtagcct tcggccagaa cttcgcggac cgcttgetca accgcaaaag    300
cctcttggtc caaatcgaac gaatacctca gcatcatggc agccgacagt atcgtagcca    360
acgggtttgc tttcccctgc cggcgatgat cgggagctga cccgtgagaa ggctcataca    420
ttctactttt ccgccaata gaggcagaag gtagcattcc caaagatccg gtcagcatag    480
aggcttcgtc ggtcaatata tctccaaaca tgttttcagt tacgatcaca tcgaattgac    540
gcggtattgc tatgagctgc atggcacagt tgctgacgta catgtggctg aatcgcagct    600
caggatactc cagagctact cgattggcca cctcgcgcca taacctagag ctttctagaa    660
cattggcctt gtccaccgat gtcactttct ttctccgttt cctcgcgccc tcgcaggcca    720
aacgaactat gcgttcgate tcatacgtcg agtactccag aacatcgata gccctttctc    780
cgcccagaag cttctcccgc cgtctctccc cgaagtacaa cccgccggtc agttccctca    840
ctaccaagag atctactccc tcgataatat cgggtttcag ggaggaagca tgaaccagtt    900
ccgggaacag gtaagcgggc cgcaggttag cgtaaagccc aagttcctta cgcagagcca    960
acagcgtctc cgctcgggc ctgagcgcag ccggcaggtt atcccatttg ggaccaccta    1020
tggctcctag aagaacagcg tcgctatctt tgcacagggc cagggtttct tcaggcaaag    1080
gaacccccac ctctctgata gccgctcccc cgaccagggc ttcggtaaaa gcgaattcgt    1140
gtttgaacct cttagcaact gctttcaata ctttttgcgc ttcaggtacg atctcggctc    1200
cgataccatc cccgggtaac acggetatct taaacctga tttagtttcg gtgtctatct    1260
cttaatagcc tcgatttatt ttcggggcta ttaatcaact ctcagaggcg acaagcttct    1320
tcttccctta cgacgttttt attggttga catggcaagg ttctctcttc tgcggtta    1378

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<210> SEQ ID NO 122
<211> LENGTH: 1327
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 122, Example
    122: designer Prochlorococcus marinus MIT9313 groE-promoter-
    controlled 3-Methylbutanal Reductase DNA construct (1327 bp)

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<400> SEQUENCE: 122
agaaaatctg gcaccacacc ccctttcaga gggcgcaac attaccactg catggcgaga    60
tcttctcagg gttcgggtgac cgcacacaggt atccactagt cggcacagca tcaacacaca    120
tagggttggc actcaatggc cacgagtgct actcatgatg tcagttttcg tttcaggtgc    180
taacgggttc attgcccac acattgtcga tctcctgttg aaggaagact ataaggtcat    240
cggttctgcc agaagtcaag aaaaggccga gaatttaacg gaggcctttg gtaacaacct    300

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aaaattctcc atggaagtgt tcccagacat atctaagctg gacgcatttg accatgtttt 360
ccaaaagcac ggcaaggata tcaagatagt tctacatacg gcctctccat tctgctttga 420
tatcactgac agtgaacgcg atttattaat tctgctgtg aacgggtgta agggaattct 480
ccactcaatt aaaaaatagc ccgctgattc tgtagaacgt gtagtctca cctcttctta 540
tgcaactgtg ttcgatatgg caaaagaaaa cgataagtct ttaacattta acgaagaatc 600
ctggaaccca gctacctggg agagttgcca aagtgaacca gttaacgcct actgtggttc 660
taagaagttt gctgaaaaag cagcttggga atttctagag gagaatagag actctgtaaa 720
attogaatta actgcccgtta acccagtta cgtttttggt ccgcaaatgt ttgacaaaga 780
tgtgaaaaaa cacttgaaca catcttgcca actcgtcaac agcttgatgc atttatcacc 840
agaggacaag ataccggaac tatttgggtg atacattgat gttcgtgatg ttgcaaaggc 900
tcatttagtt gccttccaaa agagggaac aattgggtcaa agactaatcg tatcggaggc 960
cagatttact atgcaggatg ttctcgatat ccttaacgaa gacttccctg ttctaaaagg 1020
caatattcca gtggggaac caggttctgg tgctacccat aacacccttg gtgctactct 1080
tgataataaa aagagtaaga aattgttagg tttcaagttc aggaactga aagagaccat 1140
tgacgacact gcctcccaaa ttttaaaatt tgagggcaga atataatgat ttagtttcgg 1200
tgtctatctc ttaatagcct cgatttattt tcggggctat taatcaactc tcagaggcga 1260
caagcttctt ctcccttac gacgttttta ttggttgac atggcaaggt tctctcttct 1320
gccgtta 1327

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<210> SEQ ID NO 123

<211> LENGTH: 2004

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 123, Example
123: designer Cyanothec sp. ATCC 51142 nirA-promoter-controlled
2-Isopropylmalate Synthase DNA construct (2004 bp)

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<400> SEQUENCE: 123

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agaaaatctg gcaccacacc tattaatct aaaatagctg ttttagctaa aatagtcaat 60
agcaagtctt ataggtaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca 120
aaaaacggat ttttaataca attttgttac attagctaca aaatatctca aatggtagag 180
gttaaatagg tacaactcga ccagatggag ggttttcctt gtgatgaact gttccttctt 240
cccttatact ctccctggat agaatgtagt tccttctcat gatggctctg ttaaggcat 300
ctacaaaacc gcttacgctg gcttttatta tgcctgatac cacgcctcta cctgaggctt 360
ttacattatc cagctctatc acaaggcgcg cctctgcctg cgcacccgtg ttgggggtga 420
gagcttttat agaaaagtca atgagctctg gctccacctt aagagcttct tgtatggctt 480
ttatcacage atccacagga ccggttcccg tagatgtggc agtcctttct tcacctctaa 540
agctcagcac tactgtagcg gtaggaagca ggttgctccc tgtctgaacc tgatagtgtt 600
ttacctttat aggctcttcc tctccacct tcataaactc ttcgtatatg agggcttcca 660
aatcctcate atatacctcc tttttcttat ccgcaagagc cttgaacttt tcaaagatcc 720
tctccaggtc ttcacgctt agcttaaacg caagttcatt cagtctctct ttagagcgt 780
gcctccctga gtgtttacca agtattatct tgggtggagg aaaacctaca tctcgggggt 840

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tcattatctc gtaggtgaga gggtagacca gcacaccgtg ctggtgtatg cccgattcat	900
gagcaaaaggc attatcccc actatagcct tgttgggttg aacaaaagag cccggttacc	960
tgcaaaaggag cctgctggtt ttgtatatct ctctggtgtt tatgtccgtg tagagccctc	1020
caaaagaagtc tttgcgcact ttgagagcca tcaactatctc ctcaagggtc gcgtttcctg	1080
ctctttcacc tatgcccgtg atgggtgact ctacctgtct tgcaccgtgc tttaccgcca	1140
taagggagtt ggcaacagcc atcccaaggt catcatgaca gtgcacgctt ataatggctc	1200
tgtctatggt gggcacgttg ttccttatgt cctctatgag ccttgcaaac tcttctggca	1260
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tcctctttct tetggctggc ttttagagcct cccctgctag ctctatgtcc ttttccaatg	1560
ctctggcaag ggagcatatt atcggacctt ctacctgctg tgctatcaga tggacgctct	1620
caaaagtctcc cttagatgct gctgcaaac ctgcctctat aacatccacc cccagcttgg	1680
caagttgggt agccatctga agtttttcat cagcagtcac agaaaaaacc ggcgcttgct	1740
ctcctctctc cagcgtggtg tcaaatatgt aaaccttctc cattaagctg ttttagagaa	1800
atgtgttcgg taaatattag cctacctaca gttgttgggt gtaggctaat attatgaatt	1860
gagtcctact gaaccaatga ttatcgttac gactaaaagt aataaatgct atcagcagga	1920
taggggttga taggaaaagt ttttaaatcg gatggttttc gagttagagg ttagggtttc	1980
tttaggttct ctcttctgcc gtta	2004

<210> SEQ ID NO 124

<211> LENGTH: 2648

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 124, Example 124; designer Cyanothecae sp. ATCC 51142 nirA-promoter-controlled Isopropylmalate Isomerase large/small subunits DNA construct (2648 bp)

<400> SEQUENCE: 124

agaaaatctg gcaccacacc tattaatctt aaaatagctg ttttagctaa aatagtcaat	60
agcaagtctt ataggaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca	120
aaaaacggat ttttaataca attttgttac attagctaca aaatatctca aatggtagag	180
gttaaatagg tacaactcga ccagatggag ggttttcctt gtgatggtgc caaagacgat	240
tattgaaaaa atttgggatg aacacgtggt ttaccgtgaa gatgggaaac cccgatttatt	300
atatattgat ttacatctcg ttcataaagt gacatcgcgc caagcttttg aaggattgctg	360
acaaaaagga agaaaagtgc gtcgcccaga ttttaacattt gcgacgatgg accataacgt	420
tccaacgatt aatcggtcgc ttgttgaaga tgaagtggcg aaaaatcaaa tgacagcatt	480
ggagcggaac tgcctgtagt tcggtgttcc gcttgccgat ttaaacagtc cagaacaagg	540
gattgttcat gtcacggctc cagaactcgg gttgacacag cctgggaaaa ccattgtgtg	600
tgagatagc catacgtcta cacatggcgc ttttggggca ttacgcttcg ggatcggaac	660
gagtgaagtc gaacacgtat tagcgcgcga aacgttatgg caacatcgtc caaagacgat	720

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gcaagtgaac gttacgggat cgttagcacc gggcgtatca gcaaaagacg ttattttagc 780
gatcattggc aagtttggag ttgattttgg cacaggctac gtgcttgagt ttacaggcga 840
tgttattcgt cgtatgtcaa tggaagagcg gatgacaatt tgcaatatgt cgattgaagc 900
aggggcacga gctgggttaa ttgccccaga tgatgtgacg tttgcatatt taaaagagag 960
aaaaatgca ccgaaaggag aagcgtttga gcaggcagtt gaaaagtgga agcagttatg 1020
cacagatgaa ggagcggat acgatcgtgt cgttcatatt gatggaagtg aaattgctcc 1080
aacagtgaca tggggcacia cgccagcaat gagctctccg atcgatgga ctgttccaga 1140
tccgaacgag tttgcgacag aacagagag aaaagctgta cagttagcgt tgcaatatat 1200
gggattaaag ccaggaacga aatgacgga tattgctgtg caacatgtgt ttatcggatc 1260
atgcacaaac tcgcgcataa gcgatttacg ggaagcggcg caaattgtaa aaggaaaaaa 1320
agtgcaccg ggcgtcagag cgctcgtcgt tccgggctca caacaagtaa aaaagcaggc 1380
agaagaagaa ggaattgctc aaacgtttat tgacgcaggc tttgaatggc gcgattccgg 1440
ctgtagcatg tgtcttggaa tgaatccaga tactgttcca gcaggggaac attgcgcctc 1500
aacgtcaaac cgcaatttcg aagggagaca aggaaaaggg gcgcgcacgc atctcgtgag 1560
tccagcaatg gcagcccgcg ctgcgattta cgggcatttt gtcgatgtgc gtacattgta 1620
taaagaagtg gtaagatagt attaaatcta aaatagctgt tttagctaaa atagtcaata 1680
gcaagtctta taggtaatca aacgcaacta aaatgcaaaa aatccataat taaaatgcaa 1740
aaaaaggatt ttaatacaa ttttgttaca ttagctacaa aatatctcaa atggtagagg 1800
ttaaataggt acaactcgac cagatggagg gttttccctg tgatggaacc attcgtcgtt 1860
cataaaggaa aagtgctggt cttagatcga gcaaatatag atacggatca aattattccg 1920
aaacaatttt taaaacgaat tgaacgcacc ggatttggtc aatttctttt taacgattgg 1980
cgttatttat cggacggaac accaaaacca cattttgagt taaaccgtcc tgaaaacgag 2040
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ccttgggccc ttgctgatta cggatttcgt gccattattg ctccttcatt tgctgatatt 2160
ttttacaaca actgtttgaa aaatagttta cttcctatta aacttccaaa agaagacgtc 2220
gcttatttgt taaaacaagc ggaacgggca gattacgaac taacgatttc gcttgaacaa 2280
caagtcgttt ttgatgatga agggtttaca agctcgttcg acatcgatcc gtatcgaaaa 2340
cagctccttt taaaagggtg ggacgaaatt gatttaacgt tcgtgatga accatatatt 2400
atcgcctacy aaaaaaacg ctcttgataa gctgttttag agaaatttgt tcggtaaata 2460
ttagcctacc tacagttggt gtgggtaggc taatattatg aattgagtcc tactgaacca 2520
atgattatcg ttacgactaa aagtaataaa tgtcatcagc aggatagggg ttgataggaa 2580
aagtttttta atcggatggt tttcgagtta gaggttaggg tttctttagg ttctctcttc 2640
tgccgtta 2648

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<210> SEQ ID NO 125

<211> LENGTH: 1530

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 125, Example
125: designer Cyanothecce sp. ATCC 51142 nirA-promoter-controlled
3-Isopropylmalate Dehydrogenase DNA construct (1530 bp)

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<400> SEQUENCE: 125

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agaaaatctg gcaccacacc tattaatct aaaatagctg ttttagctaa aatagtcaat    60
agcaagtctt ataggaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca   120
aaaaacggat ttttaataca attttgttac attagctaca aaatatctca aatggtagag   180
gttaaatagg tacaactcga ccagatggag ggttttcctt gtgatggcaa ccacgtatcg   240
cattgctggt ttggcggggg atggtattgg ccagaaaatt acggccgttg cccttgatgt   300
attgcgggcg atcgcccctc gctttggggt ggactttgac tttgttcccg cccttgtagg   360
gggctgccc attgatgctg tgggggaacc cttgccagca gcaacgctag ccactgtcg    420
tcagagtgat gccgtgctcc tagctgccat tggcggaaac cagtgggata gcctaccccg   480
tcactgctgc ccggaaccg gattacttgc cctgcggtct ggtctagggt tatttgccaa   540
cctacgcccc gccaaaatct ttcccagct tctccatgcc tctccctca agccggaagt   600
gattgcccgt gtggatctca tgggtgctgc cgaactgacg ggtggcattt actttggtca   660
accgcgcggt attttaccac ctgaaacggg tgagcagcgg ggggtgaata cgatggccta   720
taccgccacg gaaattgatc gcattggcgg tgttgccctt gaaaccgctc gcaaacggca   780
gggcaaaact tgctccgttg ataaggccaa tgccttgaa gtctcccaac tgtggcgcga   840
tcgctgacc gccctcagtg ctgagtaccc ggatgtgaa ctgacgcacc tttatgtgga   900
caatgcagca atgcaactgg tgcgcgcccc gaaacagttt gacacgattg tgaccagtaa   960
cctctttggt gatatactct ccgatattgc cgcctgctc accggtagta ttggcatgct  1020
tccctccgcc agcctagggg aatcggggcc agctctgttt gaaccggttc atggctctgc  1080
ccccgacatt gccggccaag acaaggccaa cccctcgcct atggtgctca gtgcccgaat  1140
gatgctgcgt tatggtctga accaaccagc ggcagcgcga gcgatcgaag aggccattac  1200
tgccgtttta gatcagggct accgcaccgg cgatttaatg tctgagggct gcacgcttgt  1260
gggctgtcgc gaaatgggca acctccta atcaaggaattg tcccgataat aagctgtttt  1320
agagaaatgt gtteggtaaa tattagccta cctacagttg ttgtggtag gctaatatta  1380
tgaattgagt cctactgaac caatgattat cgttacgact aaaagtaata aatgtcatca  1440
gcaggatagg ggttgatagg aaaagttttt taatcggatg gttttcgagt tagaggtag  1500
ggtttcttta ggttctctct tctgcgctta                                1530

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<210> SEQ ID NO 126

<211> LENGTH: 2088

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 126, Example
126: designer Cyanothece sp. ATCC 51142 nirA-promoter-controlled
2-Keto Acid Decarboxylase DNA construct (2088 bp)

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<400> SEQUENCE: 126

```

agaaaatctg gcaccacacc tattaatct aaaatagctg ttttagctaa aatagtcaat    60
agcaagtctt ataggaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca   120
aaaaacggat ttttaataca attttgttac attagctaca aaatatctca aatggtagag   180
gttaaatagg tacaactcga ccagatggag ggttttcctt gtgatgtata cagtaggaga   240
ttacctgta gaccgattac acgagttggg aattgaagaa atttttggag ttctggtgta   300
ctataactta caatttttag atcaaattat ttcacgcgaa gatatgaaat ggattggaaa   360

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tgctaatgaa ttaaatgctt cttatatggc tgatggttat gctcgtacta aaaaagctgc	420
cgcatattctc accacatttg gagtcggcga attgagtgcg atcaatggac tggcaggaag	480
ttatgccgaa aatttaccag tagtagaagt tggttggttca ccaacttcaa aagtacaaaa	540
tgacggaaaa tttgtccatc atacactagc agatgggtgat tttaaacact ttatgaagat	600
gcatgaacct gttacagcag cgccgacttt actgacagca gaaaaatgcca catatgaaat	660
tgaccgagta cttttctcaat tactaaaaga aagaaaacca gtctatatta acttaccagt	720
cgatgttgct gcagcaaaag cagagaagcc tgcattatct ttgaaaaag aaagctctac	780
aacaaatata actgaacaag tgattttgag taagattgaa gaaagtttga aaaatgcca	840
aaaaccagta gtgattgcag gacacgaagt aattagtttt ggtttagaaa aaacggtaac	900
tcagtttggt tcagaaacaa aactaccgat tacgacacta aattttggtta aaagtgctgt	960
tgatgaatct ttgcctcat ttttaggaat atataacggg aaactttcag aaatcagtct	1020
taaaaatttt gtggagtccg cagactttat cctaagctt ggagtgaagc ttacggactc	1080
ctcaacaggt gcattcacac atcatttaga tgaaaataaa atgatttcac taaacataga	1140
tgaaggaata attttcaata aagtggtaga agattttgat ttttagagcag tggttttctc	1200
tttatcagaa ttaaaaggaa tagaatatga aggacaatat attgataagc aatataagaa	1260
atttattcca tcaagtgtc ccttatcaca agaccgtcta tggcaggcag ttgaaagttt	1320
gactcaaagc aatgaacaaa tcggtgctga acaaggaacc tcattttttg gagcttcaac	1380
aattttctta aatcaataa gtcgttttat tggacaacct ttatggggtt ctattggata	1440
tacttttcca gcggttttag gaagccaaat tgcggataaa gagagcagac accttttatt	1500
tattggtgat ggttcacttc aacttaccgt acaagaatta ggactatcaa tcagagaaaa	1560
actcaatcca atttgtttta tcataataa tgatggttat acagttgaaa gagaaatcca	1620
cggaacctact caaagtata acgacattcc aatgtggaat tactcgaat taccagaaac	1680
atttggagca acagaagatc gtgtagtatc aaaaattggt agaacagaga atgaatttgt	1740
gtctgtcatg aaagaagccc aagcagatgt caatagaatg tattggatag aactagtttt	1800
ggaaaaagaa gatgcgcaaa aattactgaa aaaaatgggt aaattatttg ctgagcaaaa	1860
taaatagtaa gctgttttag agaaatttgt tcggtaaata ttagcctacc tacagttggt	1920
gtgggtaggc taatattatg aattgagtcc tactgaacca atgattatcg ttacgactaa	1980
aaagtaataa tgtcatcagc aggatagggg ttgataggaa aagtttttta atcggtgggt	2040
tttcaggtta gaggttaggg tttcttttag ttctctcttc tgcggtta	2088

<210> SEQ ID NO 127

<211> LENGTH: 1503

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 127, Example 127; designer Cyanothecae sp. ATCC 51142 nirA-promoter-controlled Hexanol Dehydrogenase DNA construct (1503 bp)

<400> SEQUENCE: 127

agaaaatctg gcaccacacc tattaatctc aaaatagctg ttttagctaa aatagtcaat	60
agcaagtctt ataggtaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca	120
aaaaacggat ttttaataca attttggttac attagctaca aaatatctca aatggtagag	180
gttaaatagg tacaactcga ccagatggag ggttttccct gtgatggaac tcgacctcga	240

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cgggtcccggg gttggtgaag tgctgatcaa gtacaccgcc gcgggggtgt gccattcgga 300
cctgcacttg accgacgggg acctaccgcc gcgctatcca atcgtcgggg ggcacgaggg 360
gtcaggcate atcgaggagc tcggacctgg ggtcaccaag gtcaaaccag gcgatcacgt 420
tgtttgcage ttcattccga actgcggaac ctgtcggtag tgcgccaccg gacgtccaa 480
cctctgcgat atggggcgcca ccattcctga aggggtgatg cccgacggca gttaccggt 540
ccacagtaac ggcttgatt tcggtgcat gtgcatgctc ggcacattct ccgaacggc 600
aactatctcc cagcattcgg tggtaagat cgacgactgg ctgccgctcg agaccgggt 660
ggtcgtcggc tgcggcgtgc cgactggctg gggcacctcc gtctatgccg gcggggttcg 720
ttgcggtgac accaccgtea tctatggcgt cggcggcctg ggagtcaacg ccgtccaagg 780
cgcggtgagt gggggcgca agtacatcgt ggtcgtcgtat ccggttgcgt tcaaacgca 840
caccgcgctc aagtteggcg ccaccacgc gttcggcgac gcccccaccg ccgcgccaa 900
ggtcgacgaa ctgacctggg gacagggtgc cgatcaggcg ctgacctgg tcggcaccgt 960
cgacgaggac gtggtctcgg cggcgactgc ggtgatcggg aaggaggca ccgtcgtgat 1020
caccggactg gcggaccag caaagctcac ggtgcacggt tcgggaacgg acctgacgct 1080
taacgagaag acaatcaagg gcacgttgtt cggtcgtcc aatccgcaat acgacatcgt 1140
acggctgctc cgtctctacg acgccggcca gctaaaactc gacgatctga tcaccaccg 1200
atacagctc gaccaggtca accagggtca ccaggatctg cgagacggca agaactccg 1260
cggcgtgatc atccacgct gataagctgt tttagagaaa tttgttcggg aaatattagc 1320
ctacctacag ttgttgggg taggctaata ttatgaattg agtcctactg aaccaatgat 1380
tatcgttacg actaaaagta ataaatgta tcagcaggat aggggttgat aggaaaagtt 1440
ttttaatcgg atggttttcg agttagagg tagggtttct ttaggttctc tcttctgccg 1500
tta 1503

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<210> SEQ ID NO 128

<211> LENGTH: 1149

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 128, Example 128: designer *Cyanotheca* sp. ATCC 51142 *nirA*-promoter-controlled short-chain Alcohol Dehydrogenase DNA construct (1149 bp)

<400> SEQUENCE: 128

```

agaaaatctg gcaccacacc tattaatct aaaatagctg ttttagctaa aatagtcaat 60
agcaagtctt ataggtaatc aaacgcaact aaaatgcaaa aaatccataa ttaaaatgca 120
aaaaacggat ttttaataca attttgttac attagctaca aaatatctca aatggtagag 180
gttaaatag tagcaactca ccagatggag ggttttcct gtgatgaagg tgccgtaat 240
tactggggca tcccgtggaa tcggggaagc tatagcaaa gcccttgctg aagatggata 300
ttcccttgcc ttaggggcta gaagtgttga taggttagag aagattgcca aggaactcag 360
cgaaaaacat ggggtggagg tattttacga ctacctgat gtatcaaaac cagaaaagcgt 420
tgaagagttt gcaaggaaaa cgctagctca ctttgagat gtggacgttg ttgtggccaa 480
tgcggggcct ggttactttg taggcttga agagcttaca gaagagcagt tccacgaaat 540
gattgaagta aaccttttgg gagtttggag aacaataaaa gctttcttaa actccttaa 600

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gcgactgga ggagtggcta ttgttgttac ttcagatggt tctgcaagc tacttccata 660
cggtggaggt tatgtggcaa ctaaatgggc tgcaagagca ttgtaagga ccttccagat 720
tgagaatcca gatgtgaggt tcttcgagct aagacctgga gcagtagata catattttgg 780
aggagcaaaa gctgggaagc caaaggagca agggatttta aaacctgagg aagttgctga 840
ggcagtaaaa tacctcctaa gacttccaaa ggatgttagg gttgaggaat taatgttgcg 900
ctcaatttat caaaaacctg agtattgata agctgtttta gagaatttg ttcggtaaat 960
attagcctac ctacagttgt tgtggtagg ctaatattat gaattgagtc ctactgaacc 1020
aatgattatc gttacgacta aaagtaataa atgtcatcag caggataggg gttgatagga 1080
aaagtttttt aatcggatgg ttttcgagtt agaggtagg gtttctttag gttctctctt 1140
ctgccgtta 1149

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<210> SEQ ID NO 129

<211> LENGTH: 1910

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 129, Example
129: a designer Nial-promoter-controlled chloroplast-targeted
Phosphoglycerate Mutase DNA construct (1910 bp)

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<400> SEQUENCE: 129

```

agaaaatctg gcaccacacc atggttaggt gcgagtgacc ccgcgcgact tgaagggtt 60
caaacgaccc cgcctacga acttttgcg gggggcgctc ccggatgta ggtgagcagt 120
gaccccgccg cacttgaag ggttcaaac accccgccgt acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgctc ccggccgctg gctcggccgg 240
cccgtccag cgtgcgccc atggccgccc tgaagcccgc cgtcaaggct gcccccgctg 300
ctgcccgcgc tcaggccaac cagatgactc gtgtcatcat tgtgcgcat ggtcaaatga 360
catataatgt tgaacgagc atccaaggac gtactgatgc gtcaacttta acggataaag 420
gtcggagcga tctgtgtaa gtgggtaaag ccctgagtaa tatagcattt acagcaatat 480
atagcagtc tctcaaccga gcgaagacga cagcggaat tattcgcagt gagttggtt 540
aacattcgtc cgtgattcag gtttctgaac atctggttga agtagattta cctttgtggg 600
caggaatggt atctcttgat gtgaaagaga agtttcctga tgactatagt atttgaaaa 660
aacgtcccca cgaattgcat atgattgtca gtgacgcaca cgggacacga gaacttttcc 720
cagttctggc tttatatgaa caagccaagc agttttggca agaaatgta tctcgtcacc 780
aaggggaaac tattctcatt gttggacata atggtattaa ccgcgctctg attagtacgg 840
ctttgggtat tcttcccagt gtttatcacg gactacaaca gtctaattgc gcgattagcg 900
ttttaaattt tgccgggtgtt ttgggtgata cggttcagct agattcaatg aatcagacgc 960
aacatttggg agatacttta cccactttgc gaccaaatca tcaaggattt agattattat 1020
tagtacgtca tggggaaaca gaatggaatc gtcaaggtaa gtttcaaggc caaattgacg 1080
ttcctctgaa tgataatggc agagcgcaag caggaaaaac tggggagttt ctccaagagg 1140
tggcgcttga ttttgccttt agtagcacta tggcgctcc aaaagaaaca gcggaaatta 1200
ttcttcagaa gcatgctgat ataaagtgg aattactaga tggtttacgg gaaatcagtc 1260
acggcagttg ggaaggcaag tttgagtcag aaatagaaca agagtttccc ggaggttgg 1320
aacgctggcg tactgtacct gctgaagtac aaatgccgca aggggaaat ttacaacagc 1380

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tatgggaacg tagtgtggct gcttggcagt caatattaca atcggctgag gtaaatcaat 1440
ggcaaatgg gttggtagtg gctcacgatg ctactaataa aactttactc tgcaatatct 1500
tgggtttate tccagaaaat ttctggaatt tccgtcaagg taatggggca gttagtgtta 1560
ttgactacce tttaggcgct agtggtttac cagtactgca agcgatgaac attactagtc 1620
at ttgagtgg tgggtgatta gataaaacgg cagcaggagc attgtagtaa atggaggcgc 1680
tcg ttgatct gagccttgc cctgacgaa cggcgggtga tggagatac tgctctcaag 1740
tgctgaagcg gtagcttagc tccccgttc gtgctgatca gtcttttca acacgtaaaa 1800
agcggaggag ttttgaatt ttgttggtg taacgatcct ccgttgattt tggcctcttt 1860
ctccatgggc gggctgggcg tatttgaagc ggttctctct tctgccgta 1910

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<210> SEQ ID NO 130

<211> LENGTH: 1856

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 130, Example
130: designer Nial-promoter-controlled chloroplast-targeted
Enolase DNA construct (1856 bp)

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<400> SEQUENCE: 130

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc cgcgcgact tggaaagggt 60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccgatggta ggtgctgagt 120
gaccccgccg gacttggaa ggttcaaacg accccgcccgt acgaactttt gtcggggggc 180
gctcccggat ggcgcgcgtc attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
cccgtcccag cgtgcgcccc atggcgcgcg tgaagcccgc cgtcaaggct gccccctgg 300
ctgccccggc tcaggccaac cagatgctta ggtcctaacc caacagtacc agcataaaca 360
gcgcatcgc ctaattcatc ttcaattctg agtaaacgat tgtatttgc taccggttca 420
ctacgacaaa gagaaccagt ttaatttga cctgcacgag tggctacagc caaatcagca 480
atagttgat cctcagtttc accagaacga tggctaatga ctgagcggaa accggttgcga 540
gtagctaaat caatagtttc caaagtttca gtcagtgaac caatttgatt caacttaac 600
aaaaatcagat tacctgcttt ttgctcaatc cctttttgta accgagtagc gttagtaaca 660
aataaatcat caccaccaa ctgtactcgt gaacccaact tctgagtcag taattgcca 720
ctttcccaat cttcctcatg taaacctatc tcaatggaaa caatcggata ttggtcaacc 780
aactggccta aataatcaat aaactcaact gggctatggg gtttaccatc ataacatac 840
tgcccatttt tgtaaaactc actcgtgccc acatccaagg ctaaagcaac ttcttcccca 900
ggcttgaac cagcttgttt aatagcagct agcaataatt ctaaagctac ttggttagag 960
tccaggttag gtgcaaaacc accttcatca ccaacaccag tcagcaaaacc cttatcatcc 1020
aaaaacttgc tgagagttgc aaaaacttcc gcaccccaac gcaaagcttc ctggaaggaa 1080
ggcgactga cgggtacaat cataaactcc tgaaaatcga cattattggc tgcgtgctct 1140
ccaccattga tcacattcat caaaggtaaa ggtagcaaat ttgctaaagg cccacccaca 1200
tagcgatata aaggaattcc caaagactca gcagcagctt tagcagctgc tagtgaaac 1260
gacaaaattg catttgcgcc caaattagct ttattgggtg aaccatcaa agagatcatg 1320
at tttatcta atgattcttg gtctagggca tccaagccta acaattgggg tgctaatacc 1380

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tctttcacat tctgcactgc cttgagtact cctttgccac cataacggct tttatcacca	1440
tctcgcagtt catgagcctc aaaagtacct gtggaagcac cgctaggaac ttgcgctagt	1500
cctactgtac cattagccaa atgtactgca gctcaacag tcggtcttcc cegtgaatca	1560
agaatttcgc gggcgataat agcttcaata gcggtatcca gaaatthtgt cattaatgg	1620
aggcgcctct tgatctgagc cttgccccct gacgaacggc ggtggatgga agatactgct	1680
ctcaagtgct gaagcggtag cttagctccc cgtttcgtgc tgatcagtct tttcaacac	1740
gtaaaaagcg gaggagtttt gcaatthtgt tgggtgtaac gatcctcctg tgattttggc	1800
ctctttctcc atggggcggc tgggcgtatt tgaagcgggt ctctctctg cegtta	1856

<210> SEQ ID NO 131

<211> LENGTH: 1985

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 131, Example 131: designer Nial-promoter-controlled chloroplast-targeted Pyruvate-Kinase DNA construct (1985 bp)

<400> SEQUENCE: 131

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaaggggt	60
caaacgaccc cgcctgacga acttttctgc gggggcgctc ccggatgga gggcgcgagt	120
gaccccgcgc gacttgaag ggttcaaacg acccgcgctg acgaactttt gtcggggggc	180
gctcccggat ggcgcgctc attgccaagt cctccgtctc cgcggcctg gctcggccgg	240
cccgtctcag cgtgcgcccc atggcgcgct tgaagcccgc cgtcaaggct gccccctgg	300
ctgccccggc tcaggccaac cagatgaaac ccttaaattt tcggactaaa attggtgcta	360
ctatcggctc tcgagtaat actccogaag tattacgtca aatgctctta gccggagtca	420
atggtgcgcg gttgaattht tcccacgcta gctacgaaga tcacgctcag atggttaaac	480
tcttacgttc tttgtccgaa gaattagact taccctgac cattttacaa gacctcaag	540
gtccaaaaat tcgggtagge aaattacccc cagacggact taacctcacc gaaggacaat	600
ctctaacctt ggttccccct gctgcttga aaaatcaagc caatacctt ggcattgatt	660
atccctacgt cgctgaagaa gcgcaaccgg gtactcaagt gctgcttgat gacggtttat	720
tggagttaac cgttgaacaa gtcaagggaa atgaggtcat ctgtcaagtg gttgaaggag	780
gcattctcaa aagcaataag ggggttaatt tgccaacct caatctacgc ttgcctcca	840
tgaccgaaaa agataagaaa gatctcgaat ttggactatc ccaaggcgtt gacatcattt	900
ccctaagctt tgtccgcaaa cccgaagata ttcaagaact caaggaattht attgcccaca	960
gatcggcaaa agttccccct tttagcgaaaa ttgaaaagcc ccaagcctt gacaatattg	1020
aagccattat cgatgaatgc gatgctatta tgggtgcgcg gggagactta ggggtagaaa	1080
tgccccccga aaaggttcca ggtatccaaa aacgcatcat taagctgtgt aaccaaaaag	1140
gcatccccct tattaccgcc acccagatgc tcgatagcat gattcgtaac ccccgtccca	1200
cccgtgctga agccagtgc gtagccaatg ctatcattga tggaaaccgat gcggttatgt	1260
tatcaggaga atcagcgatc ggagattatc ccgtgcaagc ggtgcaaatg ctggctaata	1320
ttgccaaga tattgaacca ggactgaatt ttgccaatta tcctcctcga cggcagaata	1380
aagcccacgc catagccgaa gctctcaata ccatcgacaa gattcttgat ttacaatgta	1440
ttgtcacctt tacggaacc gggattctg ctaaattagc tgctgctgaa cggccacggg	1500

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ttcccatagt ggctttaaca cctgatcacc aagtttatca tcgccttaat ttagtttggg 1560
gagtcgcgacc cattttatct gactacgatg agtcttcctt agacgacttg atggttaaag 1620
tagaagatat gttaaaaacc cgaaactacg cgacatcagg ggataaagtg ttgattatgg 1680
gtggtttacc cctcagaaaa gccagtagca cgagttttct cgatattcat acgattactt 1740
aataaatgga ggcgctcgtt gatctgagcc ttgccccctg acgaacggcg gtggatggaa 1800
gatactgctc tcaagtgtcg aagcggtagc ttagctcccc gtttcgtgct gatcagtctt 1860
ttcaacacg taaaaagcgg aggagttttg caattttggt ggttgtaacg atcctccggt 1920
gattttggcc tctttctcca tgggcgggct gggcgtatct gaagcggttc tctcttctgc 1980
cgtaa 1985

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<210> SEQ ID NO 132

<211> LENGTH: 1568

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 132, Example
132: designer Nial-promoter-controlled NADPH-dependent
chloroplast-targeted NADPH-dependent Glyceraldehyde-3-phosphate
dehydrogenase DNA construct (1568 bp)

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<400> SEQUENCE: 132

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agaaaatctg gcaccacacc atggtagggg gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgcctgacga acttttgcg gggggcgctc ccggatgta ggtgtagagt 120
gaccccgcg cacttggaag ggttcaaacg accccgcctg acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgcttc cgcggccgtg gctcggccgg 240
cccgtccag cgtgcccacc atggccgccc tgaagcccgc cgtcaaggct gcccccgtag 300
ctgccccgga tcaggccaac cagatgtcaa cgaatattgc aattaatgga atggtagaa 360
ttggaagaat ggtgctaaga atagcactaa agaatgaagc attgaatgta gttgcatca 420
atgctagcta tcctcctgaa acaatgac attttaattaa ttatgacaca acacatggga 480
gatacgataa aagagtagaa cctattgaaa gtggaattcg agtggaaagg catgatatta 540
aattagtgtc tgatagaaac ccagaaaatt taccttgtaa agatttagaa atagatatcg 600
tcattgaagc gaccggtaaa ttaaccatg gtgataaagc taaggccat attcaagcag 660
gagctaaaaa agtgttattg acaggaccat caaaaggcgg aaaagtacag atggtagtta 720
aagggttaa cgatcaagac ttagatacag atacatatga catatttagt aatgcgtcgt 780
gtactacgaa ttgtatcgga ccagttgcaa aagttttaaa tgatagtttt ggcattgaaa 840
atggcttaat gacaacggta catgcaatta caaatgatca aaataatata gataatccgc 900
ataaagatct gagaagagcg cgttctgtg gggaaagtat tataccaaca tcaacaggtg 960
ctgctaaagc attaaaagaa gttatgccag aattgaatgg caaactacat ggcatagcac 1020
ttcgtgtgcc aactcaaaat gtatcattag ttgatttagt cattgattta aaacaaaaag 1080
tgacagtaga tgaagttaat catgcattta gagatgcaaa cttacaagga attattgatg 1140
ttgaagaggc ccctctagtt tctaaggact ataatacaaa tcctcattca gcagttatag 1200
atgctaaaaa tacaatggtc atgggagata ataagggtta agttatagcc tggtagata 1260
acgaatgggg atattcctaat agagttagtg aggtagcaaa tcaacttggg gaactaatta 1320
aataataaat ggaggcgtc gttgatctga gccttgcccc ctgacgaacg gcggtggatg 1380

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gaagatactg ctctcaagtg ctgaagcggg agcttagctc cccgtttcgt gctgatcagt 1440
ctttttcaac acgtaaaaag cggaggaggt ttgcaatttt gttggttgta acgatcctcc 1500
gttgattttg gcctctttct ccatggggcg gctgggcgta tttgaagcgg ttctctcttc 1560
tgccgtta 1568

<210> SEQ ID NO 133
<211> LENGTH: 1571
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 133, Example
133: designer Nial-promoter-controlled chloroplast-targeted
NAD-dependent Glyceraldehyde-3-Phosphate Dehydrogenase DNA
construct (1571 bp)

<400> SEQUENCE: 133
agaaaatctg gcaccacacc atggtagggt gcgagtgacc cgcgcgact tgaagggtt 60
caaacgaccc cgcctacga acttttctc gggggcgcct cggatggtgta ggtgctgagt 120
gaccccgccg gacttggag ggttcaaacg accccgcccgt acgaaactttt gtcggggggc 180
gctcccggat ggccgcgcct attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
cccgtctccag cgtgcgcccc atggcgcgc tgaagcccgc cgtcaaggct gccccctgg 300
ctgccccggc tcaggccaac cagatgtaaa ctggctgaat gtacaagcat atccgcaagt 360
ttgttgagat aaccatttc gttatcgtac catgaaacta ctttcacgaa atttggagat 420
aacataattc cgcctcctt gtcgaatacc gaagtctct tctcggccac gaaatcctga 480
gaaaccacag cgtcttcagt ataccctaaa attcctttaa gttcaccttc cgatgcccgt 540
ttcattgctg cacagatttc ttcgtatgaa gtagatttct ctaactctgac cgttaaatcc 600
actacagaaa catctgcagt tgggactctg aatgacatac cggttaattt accgttaagg 660
gcaggaatta cttttctac tgcttttgca gctccggtag aagatgggat gatgttgttc 720
aatgcagaac gcccgcctct ccagtcttc atagaaggcc cgtcaacagt tttctggggt 780
gcagtagttg cgtgcacggt cgtcattaaa ccttcgatga tccgaaatt atcgtgaagt 840
acttttgcta atggagcaag acagttagtc gtacagctcg cgttagaaaa aatagtaacg 900
tcatctgtaa gatccttctg gttaacaccc attacgaaca tcggcgtatc gtcttttgaa 960
ggagcagaaa ggattgcttt ttttgacccc cgttgatat gtgcctgtgc cgcctccttg 1020
gtaaggaata aaccggttga ttccacgatg tattctgcgc ctacttcggt ccatttcagg 1080
ttgttaggat ctttttcgcg ggttacacga atctttttgc cattcacgat aaggctggtt 1140
ccttctacag aaacttcgcc cgcaaatgty cgtgtaccg agtcatactt aagcatgtac 1200
gccatatatt tggcatcgat aagatcgtta attcccacca cttcgatggt ttctctctcg 1260
gccatcgcct tgaaaaccaa gcgtccaatc ctaccgaatc cgttgattcc tactttaatt 1320
gttgacatta aatggaggcg ctcgttgatc tgagccttgc ccctgacga acggcgggtg 1380
atggaagata ctgctctcaa gtgctgaagc ggtagcttag ctccccgttt cgtgctgatc 1440
agtctttttc aacacgtaaa aagcggagga gttttgcaat tttgttggtt gtaacgatcc 1500
tccgttgatt ttggcctctt tctccatggg cgggctgggc gtatttgaag cggttctctc 1560
ttctgccgtt a 1571

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<210> SEQ ID NO 134
<211> LENGTH: 2150
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 134, Example
    134: designer Nial-promoter-controlled chloroplast-targeted
    Citramalate Synthase DNA construct (2150 bp)

<400> SEQUENCE: 134

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt    60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccggatggtg ggggtcgcagt    120
gaccccgccg gacttgaag ggttcaaacy accccgcccg acgaactttt gtcggggggc    180
gtcccggat ggccgcgctc attgccaagt cctccgctc ccggcgctg gctcggcccg    240
cccgtccag cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtgg    300
ctgcccggc tcaggccaac cagatggagc aggtttttat ctacgacacc accttgaggg    360
atggctcgca ggcagaaggt ataaactttt ccgtagagga taagatgcgc atacttcaa    420
aactggacga atttgagtg cattacatag agtgccgatg gcccggtgcg aacccaaaag    480
acactattct ctttgaagg ctgagaaaga taaaaactca aatgcccaga atagtagcct    540
ttggtgcaac aagaaaagct ggaaagaagg cgcacgaaga taagcagggt gaaaaccttt    600
tgaatcggg tgccaagtg ataaccgat ttggcaagag ctgggacttt catgtaacgc    660
atgccatag gaccacctta gaggaaaacc tggacatggt ttacgagacg gtaagctatc    720
ttaaaaagca tgtggaggag gttatctttg acgcagagca cttctttgac ggatacaggc    780
acaacgaaa ctatgctttt aaggtattgg aggcagcttt tcaggcagggt gcgactgga    840
tagtctctc gataccaac ggtggcacc ttcccaatga ggtttatgag ataacaaaa    900
aggttgtaaa aagtttcca caggcacgct taggcataca cgtccacaac gattcagata    960
ctgctgtggc taactctct atggcggctc ttgcagggtc aaggcagggt cacggcacta   1020
taaacggctt ggggaaaga acgggcaatg ctaatctgtg ttccataata cctaaccttc   1080
agctcaagct gggcttagt gtagtgctt cccaaaacct caaaaagctc accgagcttg   1140
ctcactttgt ctccgaaatc tccaacacgc cactgcccac aaacatgctt tatgtagggg   1200
agagtgcctt taccacaaa gcaggcgtac acgcctctgc agttatgaaa aggtcagaaa   1260
catacgaaca catagacct tctttgtag gaaacagaag gaagggtgaca gtgtctgacc   1320
ttctggaag gagtaatata ctttacaagc tcagggaaat ggggcttgag gtggatgata   1380
agtcccctga gcttatcaaa ctccctgaaa agataaagga acttgagaag gaaggctacc   1440
actttgaagc agctgaagct tctttgagc ttctttgcaa gaggcatttt gggcttgta   1500
aaaactattt tgacctgat gcttacaggg tgctaatagc cagaaggagt acagacctat   1560
ctcctgtttc ggaagccacc gtaagactct atgtggaaga cataaaggag catacagcag   1620
ctcttggtaa cggaccagtg agcgccttg acagaccctc cagaaaagcc ttggaagagt   1680
tttatccaag ccttaaatag gttcagctca tagactacaa ggtgagaata gttaacgaat   1740
cggagggtag atctgcaaaa gtgaggggtc ttatagaatc taccgatggt agaagaaagt   1800
ggggaacggg gggagtttcg gaaaacataa tagaagcctc ttggatagcc ttaactgata   1860
gcctcgata taaactctta aaagacgaag aagagggtat aatgtgataa atggaggcgc   1920
tcgttgatct gacccctgac ccctgacgaa cggcggtgga tggaaagatac tgctctcaag   1980

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tgctgaagcg gtagcttagc tccccgttc gtgctgatca gtcttttca acacgtaaaa	2040
agcggaggag ttttgcaatt ttgttggtg taacgatcct ccgttgattt tggcctcttt	2100
ctccatgggc gggctgggcg tatttgaagc ggttctctct tctgccgta	2150

<210> SEQ ID NO 135

<211> LENGTH: 3125

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 135, Example
 135: designer Nial-promoter-controlled chloroplast-targeted
 3-Isopropylmalate/(R)-2-Methylmalate Dehydratase large/small
 subunits DNA construct (3125 bp)

<400> SEQUENCE: 135

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaagggtt	60
caaacgaccc cgccgtacga acttttctcg gggggcgctc ccggatgta gggtgcgagt	120
gaccccgcg cacttggaag ggttcaaacg accccgcccgt acgaaacttt gtcggggggc	180
gctcccggat ggccgcccgc attgccaagt cctccgtctc cgcggccgtg gctcgcccgg	240
cccgtccag cgtgcccccc atggccgccc tgaagcccgc cgtcaaggct gccccctgg	300
ctgccccg ctaggccaac cagatgtacc atgtctctg ttgctatctc gcccttgatt	360
gctgatgctg taacagtagc cgctgaagca agatatacaa aagaatcttt atgtctgca	420
cgcccttga agtttctgt acctgtactg ataagagtct cacccacc gataaacacc	480
tgacagcttc ccagcatac agagcagtta ggattcataa caattgcacc tgcgtccatg	540
aatatatcaa ggagtcctc tttcatagcc tgaagatata ccgaacggct tgcaggaaact	600
acaaggaaatc ttaccttagg agcaaccttt ttcccttga tgatcgtgc gccaaactctt	660
aaatcctcga ttctgcccatt gttacatgaa ccaagaaatg cttcatcaat ctttacacca	720
agtgattcct tagccggaac tacattgtca acaaatgtg gctttgcaac aattggctgt	780
attgttgaaa ggtcaatata ataaacctgc tcaataactg catcatcctc tgatgtaaag	840
catgcctttg gctctctgcc atgctcctta agataatcca ttgcaacatc atcaacttcc	900
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tttcccttaa ggttgaatct taatgttccc ggaaccatta cccatgatgt tctgttaacc	1140
attgcataca aataatctgt acaaccaaca cctgtaccaa atgcacctaa cgcaccat	1200
gcacaagtat ggctgtctgc tccaaatata agctcaccgc gcactacatg attttccatc	1260
ataacctgat gacacacacc ctcgcccctg tagaacttaa tatcattagc cttagcaaag	1320
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gtcatgccat gctttgctc atctgcaggt acggctgtac tctcagactc ttctttctca	1620
ccatcaagtg atgcaataag accaccctga ttaagaatag cctgcatctt ggctggaagc	1680
ttagtacatg tataagtctt tccattaaca gttataatc catcttctaa tgaaagctca	1740

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cattcatccc	ccgcataac	ttcgtcatgg	agttctttac	atacaataac	aggaagtcc	1800
atattaatag	cattacgata	gaatattctt	gcaaatgatt	tggcaatcac	tgccctgaca	1860
cctaagtcc	taagtacgct	tggtgctgc	tctcttgatg	aaccacatcc	aaagttgtca	1920
tctgcaacaa	cgaaatctcc	cggtcttacg	gcagaagcaa	aatcagagtc	taatgattca	1980
aatgatgac	tcttcatctc	atcaattgtc	ggaacaaaa	gatactgcca	tgcaataatc	2040
tgatctgtat	caacatcttt	atcaaaactta	aatatcctac	ccatattgga	gggtgcgagt	2100
gaccccgccg	gacttggag	ggttcaaacg	accccgccgt	acgaactttt	gtcggggggc	2160
gtcccggat	ggtaggggtc	gagtgacccc	gcgcgacttg	gaagggttca	aacgacccc	2220
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cgttta						3125

<210> SEQ ID NO 136

<211> LENGTH: 2879

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 136, Example 136: designer Nial-promoter-controlled chloroplast-targeted 3-Isopropylmalate Dehydratase large/small subunits DNA construct (2879 bp)

<400> SEQUENCE: 136

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gaccccgccg	gacttggag	ggttcaaacg	accccgccgt	acgaactttt	gtcggggggc	180
gtcccggat	ggccgcgctc	attgccagt	cctccgctc	cgcgccgctg	gctcgcccg	240
cccgtcccag	cggtgcccc	atggccgcgc	tgaagcccgc	cgtaaggct	gcccccggtg	300
ctgccccggc	tcaggccaac	cagatgaatg	aactgcctta	catcagaaac	ttttccatta	360
accgcccggc	ccactaccat	cgctggaact	atcaataaag	tgcgaccggg	tgatgacccc	420
tgacggcctt	taaaattacg	gttagaagaa	gaagcactaa	tttgatcgcc	aactaactta	480

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tcagggttca	tggttaaaca	catggaacag	ccggtttctc	gccattcaaa	cccggcttgg	540
gtaaaaattt	tatcgagtcc	ttcggcttct	gcttgttgtt	taaccctttc	cgatccggga	600
acgacaaatg	ctttaacccc	tgatgctacc	tgtcttctct	gggcaaat	agccgcttct	660
cgtagatcgc	tgatgcgtcc	attggtacaa	ctaccaataa	aacagacatc	gacaggagtc	720
cccataatag	gcgatccggg	tttgagtgtc	atatattcat	aagcttcttg	agcaataaag	780
cgatcgctt	ctggtaaact	ttcaggagta	ggaaccactt	cagtcacgcc	tattccttga	840
ccgggggtaa	taccccaagt	aacagtcggt	togatctcac	cgcatcaaa	caccacgaca	900
tcatcatatt	gggcatctgc	atcactgcgg	atactcttcc	accattcgac	ggccttatec	960
cagtcttggc	ctttgggaga	aaagtctctg	cctttgaggt	attcaaaagt	cacctcatca	1020
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ccttttacc	ctaatttgcg	gatgatgtc	aggacgacat	ccttggcata	gactccgggg	1200
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ggcagatcaa	agggatgttt	tccttgcatc	tgttggcgat	catcaataaa	aacttgttct	2520
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atatcatcgc	ccactaaagg	gatacctcgt	cctgaaat	gggtgacttg	actcattaaa	2640
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gctctcaagt	gctgaagcgg	tagcttagct	ccccgttctg	tgctgatcag	tcttttcaa	2760
cacgtaaaaa	gcgaggaggt	tttgcaat	tggtggtt	aacgatctc	cgttgattt	2820

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 ggcctctttc tccatgggcg ggctgggctg atttgaagcg gttctctctt ctgccgtta 2879

<210> SEQ ID NO 137

<211> LENGTH: 1661

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 137, Example
 137: designer Nial-promoter-controlled chloroplast-targeted
 3-Isopropylmalate Dehydrogenase DNA construct (1661 bp)

<400> SEQUENCE: 137

 agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
 caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggtg ggggtcgagt 120
 gaccccgcgc gacttggaa ggttcaaacg accccgcccgt acgaaacttt gtcggggggc 180
 gctcccggat ggccgcccgc attgccaagt cctccgtctc cgcgcccggtg gctcgcccgg 240
 cccgctccag cgtgcccacc atggccgccc tgaagcccgc cgtcaaggct gcccccggtg 300
 ctgcccggcg tcaggccaac cagatgactc ggcaacaccg cataacccta cttcctggcg 360
 atggtatcgg acctgaaatt ttagccgtaa ccgtagatgt cctaaagggt ataggcaaac 420
 aattcgacct aaattttgag ttacagaag ccctcatcgg cgggtgctgc attgatgcaa 480
 ccggaacccc cttaccggaa gaaaccttaa agatttgtcg caacagtgat gcagtgcttt 540
 tagccgccat cgggggttat aagtgggata atttgcccgc tcatcaacgc ccagaaacgg 600
 gattattagg catcagagcc ggcttaggat tatttgetaa cttacgtccg gccaccattt 660
 taccgcagtt aatcgacgct tccaccctca aacgagaagt cgtcgaaggc gtggacatta 720
 tgggtgtgcg agaactcacc ggcggcattt attttggta accgaaggga atttttgaga 780
 cagaaaacgg cgaaaaacgg ggcgtgaata ccatggccta tacagaatca gaaatagacc 840
 gcattgctca aatcggcttt gaaacagccc aaaaacgtcg aggaaagctc tgttctgtgg 900
 ataaagccaa tgtcttagat gtctcccaat tatggcgcga tcgctgtaact ttaatggccg 960
 aaaaataccg agatgtagaa ctgtctcctc tctatgttga caatgcccgt atgcagctag 1020
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 cggatgcagc cgctatgta accggtagta ttgggatggt accctctgct agtttaggtt 1140
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 ataaagctaa cccgcttctg caggtactca gtgcccctat gatgtgaaa tatggcttaa 1260
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 atcgtacagg agacatcatg tcagaaggaa tgacgttagt gggatgtaag ggcattggag 1380
 aagttttgat taatgtccta gaatctttac aagggtgata aatggaggcg ctggttgatc 1440
 tgagccttgc cccctgacga acggcgggtg atggaagata ctgctctcaa gtgctgaagc 1500
 ggtagcttag ctccccgttt cgtgctgac agtctttttc aacacgtaaa aagcggagga 1560
 gttttgcaat tttgttggtt gtaacgatcc tccgttgatt ttggcctctt tctccatggg 1620
 cgggctgggc gtatttgaag cggttctctc ttctgcccgt a 1661

<210> SEQ ID NO 138

<211> LENGTH: 2174

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 138, Example
138: designer Nial-promoter-controlled chloroplast-targeted
2-Isopropylmalate Synthase DNA construct (2174 bp)

<400> SEQUENCE: 138

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt      60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta gggtgcgagt    120
gaccccgccg gacttggaag ggttcaaacg accccgccgt acgaactttt gtcggggggc    180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgccggccgtg gctcgcccgg    240
cccgctccag cgtgcccacc atggccgcgc tgaagcccgc cgtcaaggct gcccccgagg    300
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cgaatgggtg cttctcccat tgcacatgat ccttctgtga ctgacttaac cgaaaattca    540
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ccggttccaa tagccgcatc cattaattct tcgcccgttg gaccttttaa gatcacgggt    660
gctgttggaac gggcctgatc gccacatgac acttgtaact attcgagacg gaagatttct    720
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ttgcccggctc gttctcctat gccgttaata gtacattcta actgtctggc tccattttta   1260
acggcttcaa ggaagttagc caccgctaac cctaaatcat tatgtccatg aaccgagata   1320
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gagggagtta aatagcctac ggtatcggga atattaacgg tagttgctcc ggctgctatg   1440
gctctttcta atacttgata caaaaattct gggtcactac ggctgcacg tctggggaa    1500
aattctacat catctacaaa agacttcgca taagccacca tttctgggac gatttctagg   1560
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tctactccca gtcgtgccag tgcgcgagca acggttagct tctcatcaac attcagggtt   1860
gctcccgggg actgttcccc atctcggaga gtggtatcga agatgataac gcgatcgggt   1920
tgtttactca ttaaatggag gcgctcgttg atctgagcct tgccccctga cgaacggcgg   1980
tggatggaag atactgctct caagtgctga agcggtagct tagctccccg tttcgtgctg   2040
atcagctctt ttcaacacgt aaaaagcggg ggagttttgc aattttgttg gttgtaacga   2100
tcctccggtg attttggcct ctttctccat gggcgggctg ggcgtatttg aagcggttct   2160

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ctcttctgcc gtta 2174

<210> SEQ ID NO 139
 <211> LENGTH: 2882
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 139, Example
 139: designer Nial-promoter-controlled chloroplast-targeted
 Isopropylmalate Isomerase large/small subunits DNA construct
 (2882 bp)

<400> SEQUENCE: 139

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
 caaacgaccc cgccgtacga acttttctcg gggggcgctc ccggatgta ggtgctgagt 120
 gaccccgcg cacttggaa ggttcaaacg accccgcccgt acgaaacttt gtcggggggc 180
 gctcccggat ggccgcccgc attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
 cccgctccag cgtgcccacc atggccgccc tgaagcccgc cgtcaaggct gccccctgg 300
 ctgccccggc tcaggccaac cagatgcaac aactcccga catccgcaac ttcaccctga 360
 atcgccgcag tagcgaccat cgccggactc attaacaag tgcgaccgga agctgacct 420
 tgtcgtcctt taaagtctgc gttggaggag gaagcactaa tttgtctacc ttccagcttg 480
 tcggggttca tggctagaca catagaacat ccaggttcgc gccattcaaa gcctgctgct 540
 tcaaagattt tatctaaacc ttcagcttcg gcagcttttt tcaactcgttc ggaaccggga 600
 accacaaaag ccttgactcc ttccgctacg tggcgacctt tggcaatfff cgcggcttct 660
 tgcaggctac taagtctacc gttagtgcag ctaccgataa agcaaacgtc aatfffctgt 720
 cccttaatcg gttgaccagg atataaatcc atgtaacggt aagcttcttc agctacaaag 780
 cggctctctt ctaggagtcc ttctggctgg ggaatcaact gattcacacc aatacctga 840
 ccgggggtaa ttcccagggt aacagtgggg ggaatatccg cagcgttgaa tactattaca 900
 tcatcgtatt cagcatcage atcactcttg attgattccc accaagccac ggctttttcc 960
 caatcagcgc cttggggggc aaagtctcta ccttggagat aatcataggt aacttgatca 1020
 ggattgacat aaccgcatct agcggccccc tcaatggcca tgttgcagac agtcatccgt 1080
 tcttccatat tcatttctc aaaagtctga cccgctgatt cgtaggcgta acctacacca 1140
 cctttcactc ccagggtacg gatgatagc aggatgacat ctttggcata aaccccaggg 1200
 ttgagtgtgc cgttaacttc aatfffctcg actttgagtt tggataggga taaggtttgg 1260
 gaggcgagaa cgtcccgcac ttggctagta ccaataccaa aagcgcgctc cccaaacgcc 1320
 ccatgacttg aagtgtggct atcaccacag gcgacgtca ttcccggctg tgcagctccc 1380
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 atgttatttt cttgacaatt ctgctctagg gcttggatca tttctcagc caagcgcgct 1500
 acaaaaggac gcgctgatt ctctgtaggc acgatgtgat ccacagtagc cacagctccg 1560
 tcaggaaata gtaccttaa acctcgttcc cgtaacatag caaaggcttg tggactagta 1620
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 acagtgtgta agtccccaac tttatcaaac aggggtgcctt tgctcatatg gtaggggtgcg 1740
 agtgaccccc cgcgacttgg aagggttcaa acgacccccg cgtacgaact tttgctgggg 1800
 ggcgctcccc gatggtaggg tgcgagtgc cccgcccgcac ttggaagggt tcaaacgacc 1860

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ccgccgtacg aacttttgtc gggggggcgt cccggatggc cgcgcgcatt gccaaagtcct 1920
ccgtctccgc ggccgtggct cgccccggccc gctccagcgt gcgccccatg gccgcgctga 1980
agcccgccgt caaggtgccc cccgtggctg ccccggtcca ggccaaccag atgaccagca 2040
gccagtttac cccaactcac ataaggaac ttagcagatg ttaccgcac ttgctcggtg 2100
ttcgctacca actgaccgca agcatcccaa gcccagtaa taaagtgct tctggttcct 2160
tcaccaatgg agattggcgc ggtgaaatca ccaacttcta cttgcagagt ttetaagttg 2220
atgtgacat tagcttgagg attagcggct actaactctt gcaattgttt aacgatcgcc 2280
tcatcagcag tgacacaagg tacaccgatg gctacgcaat tacccaagaa aatttctgca 2340
aaactttcac caatcacgga ttgaatcccc catttagaaa ggcttgggg tgcgtgttcc 2400
cgtgaagaac cacagccaaa gttgcgggta actatgagga tatttgcgcc ttgatactgc 2460
ggttggtcaa aaggatgctc cccttttagg gctgtgcggg catcaataaa cgcgccttca 2520
cgtaacccat caaaggaat ggctttgaga taacgagcag gaataatgcg atcggtatca 2580
atatcattac ccactaaggg tatgcccgc cctgtaactt ctttaacttc actgaccatt 2640
aaatggaggc gctcgttgat ctgagccttg cccctgacg aacggcggtg gatggaagat 2700
actgctctca agtgcggaag cggtagctta gctccccgtt tcgtgctgat cagtcttttt 2760
caacacgtaa aaagcggagg agttttgcaa ttttgttggt tgtaacgatc ctccgttgat 2820
ttggcctct ttctccatgg gcgggctggg cgtatttgaa gcggttctct cttctgccgt 2880
ta 2882

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<210> SEQ ID NO 140

<211> LENGTH: 2210

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 140, Example
140: designer Nial-promoter-controlled chloroplast-targeted 2-Keto
Acid Decarboxylase DNA construct (2210 bp)

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<400> SEQUENCE: 140

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaagggtt 60
caaacgaccc cgccgtacga acttttgcg gggggcgctc ccggatggtg gggtcgagat 120
gacccccgcg gacttgaag ggttcaaacg accccgccgt acgaaacttt gtcggggggc 180
gctccccgat ggccgcgcgt attgccaagt cctccgtctc cgcggccgtg gctcgcccgg 240
cccgtccag cgtgcgcccc atggccgcgc tgaagcccgc cgtcaaggct gccccctgg 300
ctgccccgga tcaggccaac cagatgtata cagtaggaga ttacctgtta gaccgattac 360
acgagttggg aattgaagaa atttttggag ttccgtggtg ctataactta caatttttag 420
atcaaattat ttcacgcgaa gatatgaaat ggattggaaa tgctaataaa ttaaatgctt 480
cttatatgga tgatggttat gctcgtacta aaaaagctgc cgcatttctc accacatttg 540
gagtcggcga atgagtgcc atcaatggac tggcaggaag ttatgccgaa aatttaccag 600
tagtagaaat tgttggttca ccaacttcaa aagtacaaaa tgacgaaaaa tttgtccatc 660
atacactage agatggtgat tttaaacact ttatgaagat gcatgaacct gttacagcag 720
cgcggaactt actgacagca gaaaatgcc catatgaaat tgaccgagta ctttctcaat 780
tactaaaaga aagaaaacca gtctatatta acttaccagt cgatggtgct gcagcaaaag 840

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cagagaagcc tgcattatct ttagaaaaag aaagctctac aacaaataca actgaacaag 900
tgatthttgag taagattgaa gaaagthttga aaaatgccc aaaaaccagta gtgattgcag 960
gacacgaagt aattagthttt ggtthtagaaa aaacggtaac tcagthttgtt tcagaaaaca 1020
aactaccgat tacgacacta aatthttgta aaagtgcctgt tgatgaatct ttgccctcat 1080
thtttaggaat atataacggg aaactthtcag aatcagctct taaaaattht gtggagthccg 1140
cagactthtat cctaagtctt ggagtgaaagc ttacggactc ctcaacaggt gcattcacac 1200
atcattthaga tgaataataa atgattthcac taaacataga tgaaggaata atthttcaata 1260
aagthgtaga agatthttgat thtagagcag thgthttctt thttatcagaa thaaaaggaa 1320
tagaatatga aggacaataat attgataagc aatatgaaga atthattcca tcaagthctc 1380
ccttatcaca agaccgtcta thggcaggcag thgaaagtht gactcaaagc aatgaaaca 1440
tcgthgctga acaaggaaac thcattthttg gagctthcaac aatthttctta aatcaata 1500
gthcgtthtat thgacaacct thtatgggtht ctattggata tactthttcca gcgctthtag 1560
gaagccaaat thcggataaa gagagcagac acctthttatt thttgthgat gthtthcactt 1620
aactthaccgt acaagaatta ggactatcaa thcagagaaa actcaatcca atthgthttta 1680
thcataataa thgatgthtat acagthgaaa gagaatcca cggacctact caaagthtata 1740
acgacattcc aatgthggaat thctcgaat thaccagaaac atthggagca acagaagatc 1800
gthgtagtath aaaaatthgtt agaacagaga atgaatthgt gthctgthcatg aaagaagccc 1860
aagcagatgt caatagaatg thttggatag aactagthttt ggaaaaagaa gatgthccaa 1920
aattactgaa aaaaatgggt aaattatthg thgagcaaaa thaatagthaa atggagthcgc 1980
thcgtthgatct gagctthtgc cctgagcaaa cggcggthgga thgaaagatac thctctcaag 2040
thgctgaagcgt gtagctthage thccccgtht thgctgathca gthctthttca acagthaaaa 2100
agcggagggag thttgcaatt thgtthgthg thaacgathct cgtthgattt thgctcttht 2160
ctccatgggc gggctggggt thattgaaagc gthtctctct thctgctgthta 2210

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<210> SEQ ID NO 141

<211> LENGTH: 1724

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 141, Example 141: designer Nial-promoter-controlled chloroplast-targeted NADH-dependent Alcohol Dehydrogenase DNA construct (1724 bp)

<400> SEQUENCE: 141

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgctgact thgaaagggt 60
caaacgaccc cgcctacga actthttgth gggggcctc ccgagthgta gthgthcagth 120
gaccccgctc gactthgaa gthtcaaacg accccgctc acgaaacttht gthggggggc 180
gthccccgat ggcgcctc attgccaagt cctcctctc cgcggcctg gthcgcctc 240
ccccgctccg cgtgcccc atggcgcctc tgaagcccc cgtcaaggct gccccctgg 300
ctgccccgctc thaggccaac cagatggggt gctgcttctg gtatctgac gthgacgthca 360
agggtaatat cgcctthttc acccagatgg gthcatgctc gthcctcaa thgctgac 420
aggtcggthaa thcctthgct accgagthca thggcggaca gthgggthcgt cacaccaag 480
thttcaaga atthctcctc ththgctgata accgactthg thcctctc thcctthtca 540
thcgtcaggt thcataccgt thctgctac tgaagcagth thgacgctt thcctcggc 600

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cgcaccttca gcagggcagg aaataaccatg gccagcgtcc gggcgtggtc aatgtggtat 660
tttgccgtga tttcgtggcc gatcatatgc gtgctccagt catgtggcac ccttgcgccg 720
atcaggccat taagcgccaa tgtegccacc cacatgaggt tggagcgag gtcataatca 780
tcgggtgccg ccatgatacg tggcccgatt tccacaaggc tggagagcag cccttcggaa 840
aagcgatcct gcgccattcc gtccacagga taggtcatgt actgttccat cacatggacg 900
aaggagtcca caacaccgtt gataaacctgc ttcacgga ggctcatggt gcgtgtcggg 960
tcaagcacgg aaaagcgtgg atagaccagg ggattggaaa acaggagctt atccccagtg 1020
gactgccgtg aaatcacgct catgcagttc atttcagacc ccgtggcagg cagggtgacc 1080
accgtgcccc gtggcagggc cttggctcgc gctgtgcctt tgctggtcag gatgtcccat 1140
gcctcacctt catatgggac agcggcagcg acgaacttgg tcccatccat gacagagccc 1200
ccccccacgg caagcaggaa gtcgagccct tcttcacgca ccatggttac ggccttcac 1260
agggtttcat aggtgggatt ggcctcgatg ccaccgaatt cccgaaaggc cgggtaccg 1320
agggcgggcg gtacctcggc aagcgtcccg ctgctcggc cgcttgaacc gccatacagg 1380
acgaggacac gggcctgggg tgacaactga tcacttagac ggccaatcat gcctttgccg 1440
aacaggacac gtgttggtt atagaattcg aaattctgca ttaaatggag gcgctcgtt 1500
atctgagcct tgccccctga cgaacggcgg tggatggaag atactgctct caagtgctga 1560
agcggtagct tagctccccg tttcgtgctg atcagctctt ttcaacacgt aaaaagcggg 1620
ggagttttgc aattttgttg gttgtaacga tctcctgttg attttgccct ctttctccat 1680
gggggggctg ggcgtatttg aagcgtttct ctcttctgcc gtta 1724

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<210> SEQ ID NO 142

<211> LENGTH: 1676

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 142, Example
142: designer Nial-promoter-controlled chloroplast-targeted
NADPH-dependent Alcohol Dehydrogenase DNA construct (1676 bp)

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<400> SEQUENCE: 142

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaagggtt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtcgagt 120
gacccccgcg gacttggaa ggttcaaacg acccccgccg acgaactttt gtcggggggc 180
gctccccgat ggccgcgctc attgccaaat cctcctctc cgcgcccgctg gctcgcgccg 240
cccgtccag cgtgcgcccc atggcgcgcg tgaagcccgc cgtcaaggct gcccccgctg 300
ctgccccgcg tcaggccaac cagatgccta tatacttttt caaataattt ctttaaatct 360
tttaaattta cttctctgac atttccacta gtacaaatat cttcaagagc agatttagac 420
ataaatatta tttcattaa atatttttct tcatctattg ctaaatcttt tacacaacta 480
ggaagtccca agtttttatt taaaacttcc actgccacag ctaaactttc tgctccttct 540
tctgtattat ttgcaggaaa acctaaatct tttgaaatct cataatatct ttgagcagta 600
gttttatttt ctgaattgaa tctaattatg tagggtaata tagttccatt tatttttcca 660
tgagctatat gaaactttcc accaatagcg tgggctatac tatgatttat tcctaaacca 720
gatttttcaa aagcaaaacc tgctatacaa gatgcctttg ccatttcaat cctagcttcc 780

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tcacctttta tatctctata cattctcaaa agatttttaa aaataagtct tatagctgaa 840
agagcatata tttgagtata aaaatttgc tctttgcaag tgtatgactc aatagcatga 900
gtagagcat ctatactga atcagctaca actgattttg gtagtgtttt tgtaagtcca 960
ggatctagta ttgcatatc aggtatcctc tcattatctt ttaatggaat ttttacatta 1020
ttctttttat ctgtaagaac tgcataaggaa cttactctcg aacctgttcc acttgtagtt 1080
ggtaaggcta ttaaagggat agataatcca gattttttta caaaatattt aattgactta 1140
gcagatcaa gagaagaacc tcctcctatt gctaccatca catctggaag aaaatcaata 1200
accttatcta aggccttact aactatttca aatgctggat caacttcaac ttcattaaaa 1260
atcctataat ctatattttt ttgcttaaat atattttcaa attttttagt cattcctatt 1320
ttgacataa ctgaatcagt tactataaag gcttttttag ctttaatttt attaataact 1380
tcatacaatt tgtctctac ataaacattt gtatttactt caaaaatttt cattaaatgg 1440
agggcctcgt tgatctgagc cttgccccct gacgaacggc ggtggatgga agatactgct 1500
ctcaagtctg gaagcggtag cttagctccc cgttctctgc tgatcagtct tttcaacac 1560
gtaaaaagcg gaggagtttt gcaattttgt tggttgtaac gatcctccgt tgattttggc 1620
ctctttctcc atgggcgggc tgggcgtatt tgaagcggtt ctctctctcg cgtta 1676

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<210> SEQ ID NO 143

<211> LENGTH: 3629

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Coonstruct- Sequence No. 143, Example
143: designer Nial-promoter-controlled chloroplast-targeted
Phosphoenolpyruvate Carboxylase DNA construct (3629 bp)

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<400> SEQUENCE: 143

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatgga ggggctcagt 120
gaccccgcgc gacttgaag ggttcaaacg accccgcccgt acgaactttt gtcggggggc 180
gtcccccgat ggccgctgc attgccaagt cctccgtctc cgccggcctg gctcggcccg 240
cccgtccag cgtgcgccc atggccgccc tgaagcccgc cgtcaaggct gccccctgg 300
ctgccccggc tcaggccaac cagatgaccg gtatttctca ttctgcccgc aattccatta 360
atggtaata gcctctctc gagcaactct tccttagaat aacggaaatt aatgactcca 420
gactcagctt gtgcgttga ctgacgtagg cgtttgatga gagatacttg caaaaatcct 480
agaggaacaa ttgttcatt gcgtaactga acagaacgct gtaaggtggg atcgccatct 540
aggagccgtt tattctcggt aatttctaag actaagcggc aagtgcgatg atattcttgg 600
gctatttget caaacagccc ctcaaagcgt tctctatctt ccggttgaga caattccttt 660
acataatgat aggcaatttg caaatctacc ttagataagg tcatttccac ttttgagatg 720
accattttaa agaagggccca tttgagataa aaatagcgca acagtttcaa atgttcttca 780
ggttcgggtg taataaactg ttctaagcgg gttcctacc cataccaagc gggtaacaga 840
aaacgggttt gactccaact aaagacccaa ggaattgcgc gtagggact gaggtctttt 900
ttcccacttt tacgtctagc cgggcgagaa ctaatttggg gttgactaat ttcttgaatg 960
ggggtgacag acatgaagaa atccacaaaa tccggttctt cataaatcaa ggcacgatag 1020
gctttacgag aacaactggc gagttcttcc atgatctcat tccaaactcg aatatcatca 1080

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aagccgcagc ctaacaaact cgattgaata acggcagtaa caatggtttc taggttataa 1140
agggctaatt cagggagaga atatttagaa gcgaggactt ctcctgttc agtaatttta 1200
attctgecat taaccgtcgc tgcgggtga gcgagaatag cggcataagc gggccaccgc 1260
cctcgtccta ctgaaccccc gcgctcgtga aacaggcgta aatctaagcc atatctttta 1320
gcgacttttt gtaaagcttt ttgggcttta tgaatctccc aattactgct taaaaacca 1380
gaatctttgt tactgtcgga ataaccgacc ataatttctt gtaggttagt gggttctaag 1440
aggggggggt taagtgcgc cacattttcc cctgtaaag aggctaaatg atcatatcct 1500
cctgccaaag tggcgcggta aagagtcaat tcaaatagcg cccgcgatgat ttcgggtgcc 1560
cgtttaaggt cttctacggt ttcaaatagg ggtacaatgc ggatcgtgct ggaacaagtg 1620
gccgggtcat agagtcgggc ttcttgctgc aagagaagaa cctctaagac atcgtgacc 1680
tcattagtca tactgataat gtaggtatga cagatctcta aaccaaattc ttgtttagc 1740
tgccgcaaca tcctcaaggt ttctatcact tcgcaggttt tctcagaaaa cggcatttcc 1800
tggggaatga gaggacgac agtttttaat tcttcgatta accaggcgggt tcttccggct 1860
tcagtcagtt ggtttagagg tttaggcaga atttgtaaat attctgctat ttcggtgatc 1920
gcatctgagt ggttactega ctcttgccgg aaatctagtt gcgtgagggt aaaccataa 1980
acttctacct gacaaattaa gctatcta tcttgacaac ttaagccagt gcttcttagg 2040
ttacgcgcga ttaatttgag ttctctata aactcttctt tattttgga attggtggcg 2100
ttggttgtct tgaacactaa aaggcgttct tctgggttag ctaggcggct attgcggtcg 2160
cgggtatfff ccaggcgttt ttaataataa gctaacttaa gacgataggg ttcttgacgg 2220
taacgaatgg ctaactgatt ataaacttgt gggatttga cgcggtcttt ttctaggag 2280
tctaacaat ccggcaggac gttacaccaa tggagggag gactaaggat attcgataac 2340
tcatctacc tttcgatata tttttcgata acgacattgc gttgataaca ggcctggcc 2400
caagtcact ctggtgtaac aaaggggttc ccatctcgat cgcctccac ccaagacca 2460
aaataacaga aattattctt cgggtggcgt agtctgggga aggcacttt tagagttcgt 2520
ttgagacgaa gggctaattg aggaattgcc tcaaagagga cttcattaaa atagttagg 2580
gagtagtcca ctctatctaa cacggtgggt ttaaactggt gtaactcacc ggtacgccac 2640
cagagggcaa tttcttctt aagctgttct ttagcttctt ctgcttccca ggagttggt 2700
agccccattc ctctaaaggt ttctctgccc tggcttaact tttgtaaaat atgagcgata 2760
cgccgttgtt tgcgtcgaat ggtgtggcga acaatttcgg tgggatgggc tgtaaaaacc 2820
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tttaaatagg gaaatagcca gtgaaaagt cctatffff gatcattttg ttttcatcg 2940
ttcaaatctc tttctaacca atttgctcca aatgccgagg agaataact ggtttgctcg 3000
ccgttaatgc ctttttggc actggactct ccttcattgt aggtggcccg tctgagagt 3060
tgttggtctc gttgttcgta gtgtgttca acaatattaa tgagttgga atagagagca 3120
aaagcgcgag acgttcttac tgcctcattg aggtcgagtt tttcaatcaa ttgggtaatg 3180
gagttctta acgctttttg ggcttgcct tgttcagaac aaattgcacg cagttttatg 3240
agcagatcca ccaaatctt cccgcattct gcctttagca cggcttccca taaatcttcg 3300
actaatttta gtctagcttg taaaaataag tctgatgtgg agaagaattg taatgggggt 3360

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gtggggactt gaactagcga actcattaaa tggaggcgct cgttgatctg agccttgccc 3420
ctcgacgaac ggcggtggat ggaagatact gctctcaagt gctgaagcgg tagcttagct 3480
ccccgtttcg tgctgatcag tctttttcaa cacgtaaaaa gcggaggagt tttgcaattt 3540
tgttggtgt aacgatcctc cgttgatttt ggcctctttc tccatgggcg ggctgggctg 3600
atttgaagcg gttctctctt ctgccgtta 3629

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<210> SEQ ID NO 144
<211> LENGTH: 1745
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 144, Example
144: designer Nial-promoter-controlled chloroplast-targeted
Aspartate Aminotransferase DNA construct (1745 bp)

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<400> SEQUENCE: 144
agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaagggtt 60
caaacgaccc cgcctacga acttttctcg gggggcgctc ccgatggta gggtcgagt 120
gaccccgccg gacttggag ggttcaaacg accccgcccg acgaactttt gtcggggggc 180
gctcccggat ggcgcgcctc attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
cccgtcccag cgtgcgcccc atggcgcgcg tgaagcccgc cgtcaaggct gccccctgg 300
ctgccccggc tcaggccaac cagatgaacc gagacaccaa gcaggctgtt ttggaagagc 360
tgtaaccggt ccagaccctt ttcaatcgtt tggcagtcag tggcgtagga gagccggatg 420
ctgcatcgt cgccaaaggc aatgcgggga atggctgcaa cttgatgttg atccaacagt 480
tgacggcaat aggtcatcga gtcgagacct gttttgctga tgtccacgaa gacgtagaac 540
gccccttccc gtattggaca ggagagcccc gcgatttgat tcagtcctgt caagatcaac 600
tgacgcccgt ccgtaaaagg agccagcatt tctgccacac aatcctgtgg accttcgaga 660
gctgcatcgc cgcctactg ggcaaagggt cagacgtttg aggtgctgtg gctttgcagc 720
gatgcagcag cagcaattag ctcgctcgga cccgcgaggt agccaaccgc ccactcgtgc 780
atcgagttag ctttgcgcaa gccgttgcgt atcagcgttc gttcaaaaca ggcggggctc 840
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gaaacgaccc aaaagtcagt ggcttcaatg atcggcgcga tcgcttcag ttcttgccgg 960
ctatagacca tccccgtggg attggagggg gaattcagca ctagcagccg tgtccgagc 1020
gtaatcgcgc ctgccaatg ctgcccgtgg agtttaaagc cgtcgtctggc gaaagtttca 1080
acgatgacgg gcacaccgcc cgccaacttg accatttcgg gatagctcaa ccagtaaggt 1140
gcggggataa tcacctcctc gcccggtcgc agcagcacct gcatcagggt gtagagtgc 1200
tgcttaccgc cattggtgac gagaatggtg gcggcttggg aatcgagtcc gttgtcggcg 1260
cgcaattttt gggcgatcgc ttcgcgcaga tcaggttcac cggctgcagg accgtagcga 1320
gttttgcctc ctgctagcgc ttgagctgct gcattgcgaa tgtgcaaagg tgtttcaaag 1380
tcgggctccc cggcgtgtaa gctgcagaca tccaagccct cagctttcat cgctttggct 1440
tgggcagcga tcgcgagagt caacgatggt gacactcgcc ccacacgctc ggatagtttc 1500
attaaatgga gcgctcgtt gatctgagcc ttgccccctg acgaaaggcg gtggatggaa 1560
gatactgctc tcaagtgctg aagcggtagc ttagctcccc gtttcgtgct gatcagcttt 1620
tttcaacacg taaaaagcgg aggagttttg caattttgtt ggttgtaacg atcctccgtt 1680

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gattttggcc tctttctcca tgggcgggct gggcgattt gaagcggttc tctcttctgc 1740
cgtaa 1745

<210> SEQ ID NO 145
<211> LENGTH: 2366
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 145, Example
145: designer Nial-promoter-controlled chloroplast-targeted
Aspartokinase DNA construct (2366 bp)

<400> SEQUENCE: 145

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccggatgta ggtgctgagt 120
gaccccgccg cacttgaag ggttcaaacg accccgcccgt acgaactttt gtcggggggc 180
gtccccgat ggcgcgcctc attgccaagt cctccgtctc cgcggccgtg gctcgcctcg 240
cccgtccag cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtgg 300
ctgccccggc tcaggccaac cagatggcgt taattgtcca aaaatacggg ggaacctctg 360
tcggttcagt agaacgcatt caaacagttg cccaacggat tcaaaaaaca gcccaaatg 420
gcaatcaagt cgtggttgtt gtttcggcca tgggaaaaac caccgatact ttagtcaatt 480
tagccaaaga gattaccca aatccctgtc gtcgggaaat ggatatgta ttgtccacgg 540
gagaacaagt atcgatcgcc ttgatgagta tggccttaca gaaattagga caagcggcca 600
tctcctaac tggggcaca gtggggatcg tcaccgaagc agaacacagt cgagcccga 660
tcctttccat taaaccccat cgcattcaac gccatctcga tcgcggtgaa gttgtcgtgg 720
tcgcccgggt tcaaggcatt actaacgcag atgacttaga aattaccacc ctaggcggag 780
ggggctcaga taactccgcc gtcgccattg cggcagcctt aaaagccagt tgctgcgaaa 840
tctatacaga tgtccccggc atttccacca ccgatccccg catcgtcccc gatgccaat 900
taatggggga aattacctgc gatgagatgc tagagttggc cagtttaggg gctaaggttc 960
ttcatccgag ggcggtgaa attgcgcgta attatggcat tcctttagtg gtgcgatcga 1020
gttgagtgta tgcccccgga acccgcgtga cttctcccat tcctaaaccg cgatcgctag 1080
aaggcttaga actgacaaaa gccgttgatg gggtgcaatt tgacccccgat caagcaaaa 1140
tcgccttggt acgagtcccc gatcgcctcg gagtcgctgc ccgcctattt ggggaaattg 1200
cccaccagca ggtggatgta gacttaatta ttcaatcgat ccacgaaggg aatagtaacg 1260
atatgcctt tacggtgggt aaaaatgtac tcaactaaggc cgaagccgtc gctgaagcga 1320
tcgccccggc tttacggagt cattcagcga atagcgatga agcagaggta ttagtcgaga 1380
cgggagtgcc gaaaattgcc atttcagggg caggaatgat cggacggcca ggtattgccg 1440
cgaaaatggt caaaattctc gcccaagagg ggattaatat cgaaatgatc tccacctcgg 1500
aagtgaaggt cagttgtgtg attcgtcaag aagagggcga tcgcgccatt aaagccctat 1560
gccaaagggt tgaggtgaa ttgtccccga cggggattcc tgagtcagta gtagcgggtg 1620
tacctccagt tcgaggagtc gctttagatg aaaaaaacgc acaaatcgcc ctaattcatg 1680
ttcaagatcg gccgggatg gctgctagta tctttggagt cttagcggat cataacatca 1740
gtattgatac gattattcaa tcccaacgct gtcgaattgt tgagggaata cccaccctg 1800

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atatacgccct taccgttgcc caaatgatg tagaagctgc tcaaaatgcg ttaaaaaccc 1860
tagccagtgcc gtttagtgaa atgatcgctg atagcgatgt tgctaaagtc agtattgtag 1920
gggggggaat ggcgggacaa cccggggtag cggccaagtt ttttgatgct ttagctagac 1980
atcaaattaa tattaataat attgcaactt cagaaataaa aattagtgt gttgtagca 2040
aagatcaagg aattaagact ttaaaagcag ttcataaagc ctttcaatta gccggagaag 2100
aacgggtaga agttccagct taataaatgg aggcgctcgt tgatctgagc cttgccccct 2160
gacgaacggc ggtggatgga agatactgct ctcaagtctt gaagcggtag cttagctccc 2220
cgtttcgtgc tgatcagctt ttttcaacac gtaaaaagcg gagagtttt gcaattttgt 2280
tggttgtaac gatcctccgt tgattttggc ctctttctcc atgggcgggc tgggcgtatt 2340
tgaagcgggt ctctctctct ccggtta                                     2366

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<210> SEQ ID NO 146

<211> LENGTH: 1604

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 146, Example
146: designer Nial-promoter-controlled chloroplast-targeted
Aspartate-Semialdehyde Dehydrogenase DNA construct (1604 bp)

```

<400> SEQUENCE: 146

```

agaaaatctg gcaccacacc atggtagggg gcgagtgacc ccgcgcgact tggagggtt 60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccggatgta ggtgtagct 120
gaccccgccg gacttggaag ggttcaaac accccgcccgt acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcggccgg 240
cccgtccagc cgtgcccacc atggccgccc tgaagcccgc cgtcaaggct gcccccgtgg 300
ctgcccggc tcaaggcaac cagatgtcag attcatacag agtagcaata ttaggagcaa 360
ctggtgcagt aggtacggaa ttactagaat tactggaaac tcggaacttt ccagtaggag 420
aattaaaact cttagcttca gaattttcag cgggtaagac cttaaaattt aaggaacaaa 480
gtttacaagt agaagctgta acaaatgatt catttaataa agtagattta gtgctagcat 540
cagcagggtc atctgtatca aaagtatggg caaagaaagc agtagaggct ggagctgtag 600
ttattgacaa ttctagtgtc tttcgtatgg actcccagc acctcttgta gtcccagaag 660
ttaaccaga agcagcggct ttacataaag gtatagttgc taaccctaac tgcacaacaa 720
tattaatgag tgtagcagtg tggccattgc acaaaatcca gccagtcca aggttagtag 780
tgccactta tcaatcagca agtggggccg ggtcaagggc tatggcagaa atgaaaattc 840
aggccaaga aatcttagat ggaaaaactc caacaacaga tatttttccc taccattag 900
catttaattt gttccctcat aattctcaac tcaatgagca gggatattgt caagaagaaa 960
tgaaatgct tgatgaacc agaaaaat attggtctaa ggaactgaga attacagcaa 1020
cttgatttc agtaccagta ttaagagctc attcagaagc aattaatttg gaatttctg 1080
aaccatttag tgtagttaa gcacgggaag tattaagtca agcaccagga gtgacactgg 1140
tagaaaattg gcaagaaaat tattttccta tgcctatggg tgcaagtggg aaagatgatg 1200
tattggtggg gagaattcgt caggatattt ctcaagctga ggggttagaa ttatggttaa 1260
gtggagacca ggtaagaaaa ggagctgcct tgaatgcagt acaaatagct gaattattgg 1320
tggaacaaaa ttggctgaga ataccagtag gaacatttta ataatggag gcgctcgttg 1380

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atctgagcct tgccccctga cgaacggcgg tggatggaag atactgctct caagtgctga 1440
agcggtagct tagctccccg ttctgtgctg atcagtcttt ttcaacacgt aaaaagcgga 1500
ggagttttgc aattttgttg gttgtaacga tctcctgttg attttggcct ctttctccat 1560
gggcgggctg ggcgtatttg aagcggttct ctcttctgcc gtta 1604

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<210> SEQ ID NO 147

<211> LENGTH: 1868

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 147, Example
147: designer Nial-promoter-controlled chloroplast-targeted
Homoserine Dehydrogenase DNA construct (1868 bp)

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<400> SEQUENCE: 147

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta gggtgcgagt 120
gacccccgcg gacttggaa ggttcaaacg accccgccgt acgaaacttt gtcggggggc 180
gctccccgat ggccgcctc attgccaagt cctcctctc cgcgccctg gctcgcccg 240
cccgctccag cgtgcgcccc atggccgcgc tgaagccgc cgtcaaggct gcccccggtg 300
ctgccccggc tcaggccaac cagatgaagc actcgcagga cgtaggaat gctattaatc 360
gcttgagatt gctcaatttc agctaaggct tgccgaaaat tgcttctcg tacatcatga 420
gtgaccacga cgatttctgc taactgtccc tgaatccaa tttgaacgac ggattctaga 480
ctaacatggt gctgacaaa acaagttcct aaatgccaa tcaccccagg gacatcttca 540
cagagaaaagc gggcataaaa gcgagttttt aaatcttcaa ttggggtcag actacaataa 600
tgttgatggg tgacgttcaa taaaggatct aaagattgtg cttgtcctcc actgctttgc 660
agaatgccga cgatattcat aatatctgaa acgactgcac tggcgggttg acctgcccc 720
gcaccgggtc caaaaaacat cacttgtcct aacggttctc ccttgaccaa aatggcggtta 780
taaaccccat taatactggc tagtggatga tctttggcta ttaaagtggg atgtactcgc 840
acttgaagc tttctgaatc atctccttta gaacctggg caatggctaa taatttaatc 900
acaaatccga gtttatcagc ataagtaata tcggcggcac tgacttgacg aatgcctca 960
caataaatct cttcgcgttt taccgcctcg gcaaaaccga tggaggccaa aatagcaatt 1020
ttatcggtc catctaatcc gtctacatct gccgtcggat cggcttcagc atagcctaatt 1080
ttttgggctt ctgctaatac ctgcceaaaa tcggtcctct cagaggtcat ttggctgagg 1140
atataattgg tcgttccgtt aataatgcc aataatattac taatccgatt ggcccctaatt 1200
gattgtttga ggggtttaat cactggaatt cccccccca cagccgcttc taataacaca 1260
taaacgcccg ctgcattggc cgcttcataa atttcatccc cataacgagc gatcactgcc 1320
ttattggcgg tgacaatgtg ctttttatgg gcaatggcct tcatgatgag tgacttggct 1380
ggttctagtc ctccgagcag ttctaagaca atatcaatct ctggatcaat gacaatactt 1440
tctagatctg ttgtaatcac ggcggggagg agttgaactt gacgggggtt gtcaagagag 1500
cgcactcctg cccgtttaat ctcgatatct ttttaagatag gattacgtcc ccagggatcg 1560
agtagaattt gtgctgtccc cgttcccaca gttcccgaag ctaataaacc tatttttaat 1620
gccactaaat ggaggcgctc gttgatctga gccttgcccc ctgacgaacg gcgggtggatg 1680

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gaagatactg ctctcaagtg ctgaagcggg agcttagctc cccgtttcgt gctgatcagt 1740
ctttttcaac acgtaaaaag cggaggaggt ttgcaatddd gttgggtgta acgatcctcc 1800
gttgatdddg gcctctttdt ccatggggcg gctggggcgt tttgaagcgg ttctctcttc 1860
tgccgtta 1868

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<210> SEQ ID NO 148
<211> LENGTH: 1472
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 148, Example
148: designer Nial-promoter-controlled chloroplast-targeted
Homoserine Kinase DNA construct (1472 bp)

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<400> SEQUENCE: 148

```

agaaaatctg gcaccacacc atggttaggt gcgagtgacc ccgcgcgact tggagggtt 60
caaacgaccc cgcctacga actttgtcg gggggcgctc ccgatggta ggtgcgagt 120
gaccccgcg cacttgaag ggttcaaac accccgcct acgaacttt gtcggggggc 180
gtcccggat ggccgcgctc attgccaagt cctcgtctc cgcggcctg gctcggcgg 240
cccgtccag cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtg 300
ctgcccggc tcaggccaac cagatgatta acaatdddgg ccccttccat atctaaagag 360
agtgttgca cttgcgcgc tacccttca ttttcccac ccacctcat cgettcagtc 420
accctatccg cttcaccctg agaacttaaa gcgagtaaag tgggacctgc cccactgatt 480
accataccat aagccccgc tcgatagacc gctcgtttca ccgcttcgta accttaatt 540
aaacctgac gataggggtg atgcagctta tctccatcg ccatagctaa ccagtctct 600
cgcccctgt cgagtccttg taacaataac cctaacctg caatattaaa aatagcatca 660
gcgcgctat actgagtggt taaaacgcct ctcgcttct gagtagataa tcaaaaatcc 720
ggaatagcca caatgggat cagttgtca taccaggaa tctcgcaaat ttgccaattc 780
cccattccc ccacacataa gcgactgctt cccaataaag cgggaaccac attatcgga 840
tgtccttcta aagatatgac taattccatc acttcagatt gagttaaagg gttaccggct 900
agataattdg cccccactaa accccctaca atggctgtgg ctgaacttc taaccctctg 960
gctaaaggaa ccctaattt gatctcaatt tctacagcag gaaccggtg attgagatgt 1020
tgatagaaaa gcgcaaaaga ttgataaagt aaattagttt tatctcgact aaccgcttct 1080
gcttctgcgc cgcttacgag aattdtctc tcggttdctg aagtgagagt aaacttaaat 1140
tgattataaa gggttaaac ggctcctaga caatcaaagc ctggaccaag attagcggta 1200
gtagcaggaa cggttagggt aacggtcatt aaatggaggc gctcgttgat ctgagcctg 1260
ccccctgacg aacggcgggt gatggaagat actgctctca agtgctgaag cggtagctta 1320
gtccccggt tcgtgctgat cagcttdttd caacacgtaa aaagcggagg agtdttdgca 1380
tdttdttdg ttgtaacgac ctcgcttgat ttdggcctct tctccatgg gcgggctggg 1440
cgtattdgaa gcggttdctc cttctgcctg ta 1472

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<210> SEQ ID NO 149
<211> LENGTH: 1655
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 149, Example

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149: designer Nial-promoter-controlled chloroplast-targeted
Threonine Synthase DNA construct (1655 bp)

<400> SEQUENCE: 149

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaagggtt    60
caaacgaccc cgcctacga acttttctcg gggggcgctc ccggatggta ggggtcgagt    120
gaccccgccg cacttgaag ggttcaaacg accccgccgt acgaactttt gtcggggggc    180
gtccccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcggccgg    240
ccccgtccag cgtgcgcccc atggccgcgc tgaagcccgc cgtcaaggct gcccccgctg    300
ctgccccggc tcaggccaac caggtgaccc taacttgcc tatttctaaa tctgcgtcct    360
ggtcagggtc gatcaacgcc taccttctc atttacctgt cacggaagcc actccgattg    420
tcacctgca tgaagggaa acgcccctga ttccgggtgc cagcattgcc gctgaaattg    480
gccggcaagt tcaggctcat gtcaagtacg acggtttgaa cccaccgggc agctttaaag    540
atcgggggat gaccatggcg atctccaagg ctaaggaagc cggagccaag gcggtgatct    600
gtgccagtac cggcaatacc tctgcggcgg cagcggccta tggacggcgg ggcggcatgc    660
gggtctttgt cctcatcccc gatggttatg tcgctctggg aaaattagcc caagccctgg    720
tctatggcgc agaggtgctg gccattcagg gcaactttga tcaggcattg accctggctg    780
agcaattagc cgaaccaccg cccgtcaccg tggtaattc cgtcaacccc taccgctgg    840
aaggtcagaa aactgctgcc tttgaagtgg tggatgcctt gggtaatgcc cccgactggc    900
tctgtattcc cgtgggcaat ggccgcaata tcaccgctta ctggatggga ttctgtcagt    960
atcgggaaca ggatcgttgc gatcgtctac cccggatgat gggttttcaa gcagccggct   1020
ctgctcccc tgtccatggc caggtggtga cccatcctga aactgtagcg accgccattc   1080
ggattggtaa cccggccaac tggcagcggg cgatggccgt gccggatgcc agccagggag   1140
aattcaatgc tgtcagcgat gccgaaattc tcgctgccta ccgtctgctg gccagtcagg   1200
aagggatctt ttgtgaaccc gccagtgcgc cgtccgtcgc cggctctatta aagggtgaaag   1260
atcaggttcc gacgggggca acggtggtct gtgtcctgac ggggaatgga ttgaaagatc   1320
ctgatagcgc aattaagcag caaagtaacc agttccatca gggcatccca gctcagctcg   1380
aagccgtggc agccgtgatg ggcttccgct agtaaatgga ggcgctcgtt gatctgagcc   1440
ttgccccctg acgaacggcg gtggatggaa gatactgctc tcaagtctg aagcggtagc   1500
ttagctcccc gtttctgctg gatcagctct tttcaacacg taaaagcgg aggagttttg   1560
caattttgtt ggttgaacg atcctccgct gattttggcc tctttctcca tgggcgggct   1620
gggcgtatct gaagcgggtc tctcttctgc cgtaa                                1655

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<210> SEQ ID NO 150

<211> LENGTH: 2078

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 150, Example
150: designer Nial-promoter-controlled chloroplast-targeted
Threonine Ammonia-Lyase DNA construct (2078 bp)

<400> SEQUENCE: 150

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaagggtt    60
caaacgaccc cgcctacga acttttctcg gggggcgctc ccggatggta ggggtcgagt    120

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gacccccgcg cacttggaag ggttcaaacg accccgcgct acgaactttt gtcggggggc 180
gtccccgat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcgcccg 240
cccgtccag cgtgcgcccc atggcgcgct tgaagcccgc cgtcaagget gcccccggtg 300
ctgccccgpc tcaggccaac cagatgtaag aagagtcgat aggctggatt gtcactttcg 360
ttccagtacc gatagcccag ggttttgaga aatgattccc actgagccat ctcttggtga 420
gggacttgca tcccgacgac gattctgccc tagtctgccc cgtgattgcg gtagtggaat 480
aggctgatgt tccaatctgg gctcattttc gtgacaaaagt tagctaagtc gcccggtcgc 540
tcaggaaatt caaacggata gagcagttcg ttttgageta gcggtgactt gccgcctacc 600
atgtgccgca aatgcatctt ggctagctca tcatcagtta gatctagcgt cttaaatcca 660
ttgtctctaa acttggtggc gatcgcctgc gcacggctc tattttctat ctgaatgccc 720
acaaaaatat gagcttgatc ggcatctgca atccgatagt tgaattcgtc aaggctgtgg 780
ctaccgagga tatcgcaaaa tttgctgaga ctgcctgccc gctctggaat cgtaacggca 840
aagacggcct ctcttctctc tcttagctcg gctcgcctcg ctacaaaagc cagacgatca 900
aagttcatat tggccccaca agcagtcgcc accaacgttt ctccctgaat ttcttctcgc 960
tctacatagg ctttgatacc tgccatcgcg agcgcgccag cgggttccaa aatgagcga 1020
gtatcttcaa atacgtcctt gatcgcggcg caggtatcat ctgtagttac cagcaacaca 1080
tcatcgacat actggtggca cagacgaaac gtttctctcc cgacctggcg caccgctacg 1140
ccatctgcaa acaggcctac ttgatccaac ttgacacgat tcccttttgc taatgattga 1200
cgcatggcat cagcatccac cggctccaca ccaatgatct taacttcagg acgtaagcgt 1260
ttgatataag cggcaatgcc cgaaattaa ccaccccccc caatcgcaac aaaaatggca 1320
tgaatcggct tctgatgctg tcgcaagatc tccatgccga tcgttccctg cccggcaatg 1380
acgtctggat cgtcaaacgg atgcacaaa gtaagtcctt tttccaactc tagctggcgt 1440
gcgtgtgcat aggcacgctc gtaggtgtca ccgtgtaaga caacgagtc gcctctggcc 1500
ttaccgcat caatcttgac ttgcccgcgc gtgatcgcca taacaattac cgctgacgta 1560
cccaattctc tagcgcctag cgcaccccc tgtgcgtggg tgcccgcaga agctgcaatc 1620
accccacgct gcaacagttc tgaaggtagc tgcgccattt tgttataggc accgctagt 1680
ttgaaggaga aacagactg tacatcttcc cgttttagca gcacctgatt acctagccgc 1740
tccgatagcc tcggcgcgat atctaaagcc gtttcttgcg ccacgtcata aacgcgggpc 1800
ttcaaaatgc gttctaaata gtcagaataa accactaaat ggaggcgctc gttgatctga 1860
gccttgcccc ctgacgaaac gcggtggatg gaagatactg ctctcaagtg ctgaagcggc 1920
agcttagctc cccgtttcgt gctgatcagt ctttttcaac acgtaaaaag cggaggagtt 1980
ttgcaatfff gttggttga acgatctccc gttgattttg gcctctttct ccatggggcg 2040
gctgggcgta tttgaagcgg ttctctcttc tgccgtta 2078

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<210> SEQ ID NO 151

<211> LENGTH: 2282

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 151, Example 151: designer Nial-promoter-controlled chloroplast-targeted Acetolactate Synthase DNA construct (2282 bp)

<400> SEQUENCE: 151

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt	60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccgatggta gggtcgagt	120
gaccccgccg gacttggaa ggttcaaacg accccgcctg acgaaacttt gtcggggggc	180
gctcccggat ggccgcctc attgccaagt cctccgtctc cgcggccgtg gctcggccgg	240
cccgtccag cgtgcgcccc atggcgcgc tgaagccgc cgtcaaggct gccccgtgg	300
ctgccccg ctaggccaac cagatgttga caaaagcaac aaaagaacaa aaatccctt	360
tgaaaaacag agggcgagg cttgtgttg attgcttagt ggagcaagg gtcacacatg	420
tatttggcat tccaggtgca aaaattgat cggtatttga cgctttaca gataaaggac	480
ctgaaattat cgttgcctg cacgaacaaa acgcagcatt catggccca gcagtcggcc	540
gtttaactgg aaaaccggga gtcgtgttag tcacatcagg accgggtgcc tctaacttg	600
caacaggcct gctgacagc aacctgaag gagaccctgt cgttgcgctt gctggaacg	660
tgatccgtgc agatcgttta aaacggacac atcaatcttt ggataatgcg gcgctattcc	720
agccgattac aaaatacagt gtagaagttc aagatgtaaa aaatataccg gaagctgtta	780
caaatgcatt taggatagcg tcagcaggcc aggctggggc cgcttttctg agctttccgc	840
aaatgttgt gaatgaagtc aaaaatacga aaaacgtgcg tgctgttga gcgcaaac	900
tcggtcctgc agcagatgat gcaatcagtg cggccatagc aaaaatcaa acagcaaac	960
ttctgtcgt tttggtcgc atgaaaggcg gaagaccgga agcaattaa gcggttcgca	1020
agctttttaa aaaggttcag cttccatttg ttgaaacata tcaagctgcc ggtaccctt	1080
ctagagatt agaggatcaa tttttggcc gtatcggttt gttccgcaac cagcctggcg	1140
atttactgct agagcaggca gatgtgttc tgacgatcgg ctatgaccg attgaatatg	1200
atccgaaatt ctggaatgc aatggagacc ggacaattat ccatttagac gagattatcg	1260
ctgacattga tcatgcttac cagcctgatc ttgaattgat cggtgacatt ccgtccacga	1320
tcaatcatat cgaacacgat gctgtgaaag tggaaattgc agagcgtgag cagaaaatcc	1380
tttctgattt aaaacaatat atgcatgaag gtgagcagg gctgcagat tggaaatcag	1440
acagagcgca ccctctttaa atcgttaaag agttgcgtaa tgcagtcgat gatcatgtta	1500
cagtaacttg cgatcctgct tcgcacgcca tttggatgct acgttatttc cgcagctacg	1560
agccgttaac attaatgatc agtaacggta tgcaaacact cggcgttgcg cttccttggg	1620
caatcggcgc ttcattggtg aaaccgggag aaaaagtggg ttctgtctct ggtgacggcg	1680
gtttcttatt ctcagcaatg gaattagaga cagcagttcg actaaaagca ccaattgtac	1740
acattgtatg gaacgacagc acatatgaca tggttgcatt ccagcaattg aaaaaatata	1800
accgtacatc tcgggtcgat ttcggaata tcgatcctg gaaatagcg gaaagcttcg	1860
gagcaactgg cttgcgctga gaatcaccag accagctggc agatgttctg cgtcaaggca	1920
tgaacgctga aggtcctgct atcatcgatg tcccggttga ctacagtgat aacattaatt	1980
tagcaagtga caagcttccg aaagaattcg gggaaactcat gaaaacgaaa gctctctagt	2040
aaatggaggc gctcgttgat ctgagccttg cccctgacg aacggcggtg gatggaagat	2100
actgctctca agtgetgaag cggtagctta gctccccgtt tcgtgctgat cagtctttt	2160
caacacgtaa aaagcggagg agttttgcaa tttgttggg tgaacgatc ctcgctgat	2220
tttggcctct ttctccatgg gcgggctggg cgtattttaa gcggttctct cttctgctg	2280

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 ta 2282

<210> SEQ ID NO 152
 <211> LENGTH: 1562
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 152, Example
 152: designer Nial-promoter-controlled chloroplast-targeted Ketol-
 Acid Reductoisomerase DNA construct (1562 bp)

<400> SEQUENCE: 152

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggagggtt    60
caaacgaccc cgcctacga actttgtcg gggggcgctc ccggatgta ggtgcgagt    120
gaccccgcg cacttgaag ggttcaaac accccgcct acgaacttt gtcggggggc    180
gtccccgat ggccgcctc attgccaagt cctccgttc cgcggcctg gctcgcccg    240
cccgtccag cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtg    300
ctgccccgc tcaggccaac cagatgagc ttttttagc aactaaacat agctcgtaaa    360
tctttaccga cttctcaat aggatgttc gcttcttgac gacgatggc gataaatccg    420
ggttttctg actggtttc taagacaaa tcccagcaa attgaccgga ttgaatttct    480
ttaaggattt tccgcatttc ttgccgggt tgatccgtaa caatacgagg acctctagta    540
tagtctccat attctgccg attggaaatg ctatcgcca tcttagetaa tccgcctct    600
accaccagat caacgattag ttaacttcg tggagacatt caaataaag caattcaggc    660
tgatatccg cttcgactaa ggtttcaaat ccgctttaa ttaaagcact tagaccgcca    720
cagaggacta cttgttacc aaataaatca gtttctggtt cttctcgaa agtcgtttct    780
aaaaacccg cagagttcc tccaatcct ttagcataag ccatagcgcg atcgcgggct    840
tgctcgaag catctgaaa gactgcaaat aaacagggga ctcttcgcc ttgggtgtag    900
gtacgtctga cgagatgtc tggaccttt ggtgccacca taaccacatc taccgtagaa    960
ggaggaatta cttgtccaaa atgaatatta aatccatgag caaacaaaag aactttgcct    1020
tcttttaaat ggggttcaat ttcattttta tagacgcttt tttgtacctc atctggcagc    1080
aaaatcataa tccagtcgac ggccggcggc gcacgggcta cacttttaac cgttaagccg    1140
gcttcagtgg ctttttgggc tgacttactc ccaggataca gccccacaat aacattaact    1200
ccgctatctt taagattaag ggcattggca tggccttgag aacatagcc gataatggca    1260
accgttttat tagcaagtaa gtctaaatg gcactttcat cgtaatacat tcgagccatt    1320
aatggaggc gctcgttgat ctgagccttg cccctgacg aacggcggtg gatggaagat    1380
actgctctca agtgctgaag cggtagctta gctccccggt tcgtgctgat cagtctttt    1440
caaacgtaa aaagcggagg agttttgcaa tttgttggg tgtaacgatc ctccgttgat    1500
tttggcctct ttctccatgg gcgggctggg cgtattttaa gcggttctct cttctgccgt    1562
  ta 1562
  
```

<210> SEQ ID NO 153
 <211> LENGTH: 2252
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 153, Example
 153: designer Nial-promoter-controlled chloroplast-targeted
 Dihydroxy-Acid Dehydratase DNA construct (2252 bp)

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<400> SEQUENCE: 153

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt      60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtcgagt    120
gaccccgcgc gacttggaa ggttcaaacg accccgccgt acgaactttt gtcggggggc     180
gctcccggat ggccgccgctc attgccaagt cctccgtctc cgcgcccggtg gctcgcccgg   240
cccgtccag cgtgcccacc atggccgcgc tgaagcccgc cgtcaaggct gcccccggtg    300
ctgccccggc tcaggccaac cagatgtcag ataattacag aagtcgcac attacacaag     360
gcagtcaacg tacaccaaac cgggcccagc ttcggggcgt aggttttggg gataatgact    420
ttactaagcc cattgtggga gtggctaacg gatacagcac cattaccctc tgtaatatgg     480
gcatcaatga tctcgcgctg cgggcogaag ccggactcaa gcaagcggga gccatgccgc    540
aaatthtcg caccattacc gtgagtgcg gaatthtctat gggaacagaa ggaatgaaat     600
actccctcgt ctgcccggat gtgatcgctg actctatcga aaccgcttgt aacggtaaaa    660
gtatggatgg tgtgcttgcc attggcggct gtgataaaaa tatgcccggg gccatgatcg    720
ccatcgctcg tatgaatacc cctgctatct tcgtctatgg cggtagcatt aaaccggaa     780
aatacaacgg acaagattta accgttgtca gtgcctttga agccgtagga caacatagtg     840
ccggtaaaat agatgatgct caattattag gaatagaacg gaatgcttgc cccggggcgg     900
gttccctgtg gggaatgttt accgctaaca ccatgtctc cgctttttaa gtaatgggga    960
tgagcttacc ttattcttcc actatggcag cagaagatgc agaaaaagcc gacagtaccg   1020
aaaaatccgc ttttgtgctg gtagatgcca tcagaaagca aatthtgccc agtcagattt    1080
taaccggtaa agcctttgag aatgcgattt ccgtgattat ggccggtggg ggatcgacca   1140
acgcggtttt acatttatta gcgatcgctc ataccatagg ggtagaactg agcatcgatg    1200
actttgaagc cattagagct agagttccc tacttttga cctcaaacg agtggacgct     1260
atgtcatcgt tgatttcat cagggcgggg gcattcccc agtgatgaaa atgcttctcg    1320
tccatgactt attacacggg gatgctttaa ccatcaccgg tcaaacgggt gcagaagttt    1380
taaaagacgt acccgatgaa cccctcaag gacaagatgt cattcgtcct tggaaataacc   1440
cagtgataaa agaaggacac ctagegatct taaaaggaaa tttagccacc gagggagcag    1500
tcgctaaaat tagcggggtc aaaaatccta aaattaccgg tccggcgcgga gtatttgaat   1560
ccgagggaaa ctgtctagag gcgattcttg caggtaaaat tcaagctggc gatgtgatta    1620
ttgtctgta tgaagggcc aaagtggtcc ccggtatgag agaaatgta gccccgactt    1680
cagccattat tggggcagga ttgggagatt ccgtaggatt aattactgat gggcgttttt    1740
ctgggggaaac ttacgggcta gtcgtcggtc atgttgcccc agaagcagca gtaggcggaa   1800
atattgccct cgtacaagag ggagatagca ttaccattga tgcgaaagag cgattattac    1860
agcttaatgt agctgaagat gaattaatcc gtcgtcgcgc taactggcaa cgcgccatcc   1920
ctcgttatac caaagggtga ttagcgaaat atgccaaatt agtctcttct agtagtatag    1980
gagcggttac cgacaaagat ttattctaataaatggaggc gctcgttgat ctgagccttg    2040
ccccctgacg aacggcgggt gatggaagat actgctctca agtgctgaag cggtagctta    2100
gctcccctgt tcgtgctgat cagctttttt caacacgtaa aaagcggagg agttttgcaa   2160
ttttgttggg tgtaacgata ctcggttgat tttggcctct ttctccatgg ggggctggg    2220

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 cgtatttgaa gcggttctct cttctgccgt ta 2252

<210> SEQ ID NO 154
 <211> LENGTH: 1496
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 154, Example
 154: designer Nial-promoter-controlled chloroplast-targeted
 2-Methylbutyraldehyde Reductase DNA construct (1496 bp)

<400> SEQUENCE: 154

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaagggtt    60
caaacgaccc cgcctacga actttgtcg gggggcgctc ccggatgta ggtgcgagt    120
gaccccgcgc gacttgaag ggttcaaac accccgccgt acgaactttt gtcggggggc    180
gtcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcgcccgg    240
cccgtccag cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gccccgtgg    300
ctccccggc tcaggccaac cagatggcag ttccatcaa ttctactaaa actttcaagc    360
tgaacaacgg cctgtccatt ccagcagtcg gtctcggaac atggcaatcc accgatgaag    420
aggottacaa tgccttatt gctgcattga aagctggata cagacacatc gacacagctt    480
actgttacgg aatgaagag ccaattggca aagctattaa ggactctgga gttgcaagaa    540
aggacatttt tattactacc aaactttggg gaactgacca caccagaact gaggaagggt    600
tggataggtc tctgaaattg ttgggtttgg actatgttga tctgttcttg atgcattggc    660
cagttccaat gaacccta atggaaaccac acaaatttcc tacattacca gacggtaaac    720
gtgacattct gtttgactgg aacttogttg atacatacag agagatgcaa aaattagttg    780
cctctggaaa gaccaaggca atcgggtgtg ccaatttttc tatcactaac ttgaagaaat    840
tgcttcgaga ccagaaatc accatcaagc cagttgtcaa ccaagttgaa attcaagggt    900
atctgcgcga gcagagactt ttggagtatg cgaaggaaaa tgatattgtt ttggaggcat    960
attcacctgt gggatccact ggtgccccat tgctgaaaga tgagctgggt caggacctag    1020
ccaagaagaa tggatattct gaactactc tottaatttc ctgggcagtg tggagaggta    1080
tcgtcgtttt accaaaaatc gtaacgcctt ccagaattgc tgataatctt aagatcattg    1140
agttgtgtga ggaggatgga aaaaaactta atgaattggc ctcgattaga ggagaaaaac    1200
gattagttag ccctccttgg gatcctattg tcgtcttcaa cgatgaagac taataaatgg    1260
aggcgctcgt tgatctgagc cttgcccctt gacgaacggc ggtggatgga agatactgct    1320
ctcaagtgct gaagcggtag cttagctccc cgtttcgtgc tgatcagtct tttcaacac    1380
gtaaaaagcg gaggagtttt gcaattttgt tggttgtaac gatcctcctg tgattttggc    1440
ctctttctcc atgggcgggc tgggcgtatt tgaagcgggt ctctcttctg ccgtta    1496
  
```

<210> SEQ ID NO 155
 <211> LENGTH: 1595
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct- Sequence No. 155, Example
 155: designer Nial-promoter-controlled chloroplast-targeted
 3-Methylbutanal Reductase DNA construct (1595 bp)

<400> SEQUENCE: 155

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tgaagggtt 60

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caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtgcgagt 120
gaccccgcgc gacttgaag ggttcaaacg accccgcegt acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcgcccgg 240
cccgtccag cgtgcgcccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtag 300
ctgccccggc tcaggccaac cagatgtcag ttttcgttcc aggtgctaac gggttcattg 360
cccaacacat tgtcgatctc ctggtgaagg aagactataa ggatcatcgg tctgccagaa 420
gtcaagaaaa ggccgagaat ttaacggagg cctttggtta caacccaaaa ttctccatgg 480
aagttgtccc agacatatct aagctggagc catttgacca tgttttccaa aagcacggca 540
aggatatcaa gatagttcta catacggcct ctccattctg ctttgatctc actgacagtg 600
aacgcgattt attaattcct gctgtgaacg gtgttaaggg aattctccac tcaattaaaa 660
aatacgcgca tgattctgta gaacgtgtag ttctcacctc ttcttatgca gctgtgttcg 720
atatggcaaa agaaaacgat aagtccttaa catttaacga agaactcctg aaccagcta 780
cctgggagag ttgccaaagt gaccagtta acgcctactg tggttctaag aagtttgctg 840
aaaaagcagc ttgggaattt ctaggagaga atagagactc tgtaaaattc gaattaactg 900
ccgttaacce agtttacggt tttggtccgc aaatgtttga caaagatgtg aaaaaacact 960
tgaacacatc ttgcgaactc gtcaacagct tgatgcattt atcaccagag gacaagatac 1020
cggaactatt tgggtgatac attgatgttc gtgatgttgc aaaggctcat ttagttgcct 1080
tccaaaagag ggaaacaatt ggtcaagac taatcgatc ggaggccaga tttactatgc 1140
aggatgttct cgatattcct aacgaagact tccctgttct aaaaggcaat attccagtgg 1200
ggaaaccagg ttctggtgct acccataaca ccctggtgct tactcttgat aataaaaaga 1260
gtaagaaatt gttagtttcc aagttcagga acttgaaaga gaccattgac gacactgcct 1320
cccaaathtt aaaatttgag ggcagaatat aataaatgga ggcgctcgtt gatctgagcc 1380
ttgccccctg acgaacggcg gtggatgaa gatactgctc tcaagtctg aagcggtagc 1440
ttagctcccc gtttcgtgct gatcagctt tttcaacacg taaaaagcgg aggagttttg 1500
caatthttgt ggttgaacg atcctcgtt gatthttgct tctttctcca tgggcccgt 1560
gggcgtatth gaagcgggtc tctcttctgc cgtta 1595

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<210> SEQ ID NO 156

<211> LENGTH: 1739

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 156, Example 156: designer Nial-promoter-controlled chloroplast-targeted NADH-dependent Butanol Dehydrogenase DNA construct (1739 bp)

<400> SEQUENCE: 156

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggagggtt 60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta ggggtgcgagt 120
gaccccgcgc gacttgaag ggttcaaacg accccgcegt acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcgcccgg 240
cccgtccag cgtgcgcccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtag 300
ctgccccggc tcaggccaac cagatgaata atthtacttt ttataatcct actaaaatta 360

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actttggtag aggagttgaa tcctctgttg gagaagagat taaaagctta ggagctagta 420
aagtgccttt tcattatggt ggaggaagta ttgaaaaaa tgggttatat gatagaattt 480
tagattcttt aaataaagca ggattacatg taatagaact tggaggagtt aagcctaac 540
ctagattaag cttagttaaa aagggaaatag aactttgtaa aaatagtaaa gttgatttta 600
tacttgcaatg tggaggagga agtgttattg actcagctaa agccatagct cttgggttcc 660
cttatgatgg agatgtatgg gatttcttta ctaaaaatat taaaatagaa aaagctttac 720
cattaggtac agtattaaca ataccagcgg caggaagtga atcaagctca ggaactgtta 780
taactaatga agatggatgg tataagagat caacaggatc accacttcta taccctaaat 840
tttcaatggt aaatccagaa ctatgcttta ctttaccaga atatcaaata gcatcaggaa 900
gtgcagatat tttagcacat ttaatggaaa gatatttcac aaacacaaag aatggtgaac 960
ttatagatag attaatgtag ggaacaatga aaacagtaat aaataatggt cctaaggat 1020
taaaaaataa ggaagactat gatagctttg cagaggttat gtgggctgga acaatagcac 1080
ataataatct tttaaagcaca ggaagagaaa cagattgggc atcacataat atagaacatg 1140
aattaagtgg aatataatgat gttactcatg gtgcaggatt agctgttata tcccagctt 1200
ggatgaagtt tgtttataag catgatctag atagattcaa ccaatttgc actagagtat 1260
ttgatgttca agttgaagat aaaactaagg aagagggtgc cttagaagga attaagaaac 1320
ttgaagaatt cttcaaatca ataaatcttc cagtaacttt aaaagagtta gaaatagggtg 1380
aagatagatt agaagaaatg gctaaaaaat gcacagataa tgatgagcat acagtaggtc 1440
atttttaga attaaacaca gaggacatcc ttgaaatata caaattagct ttatagtaaa 1500
tggaggcgct cgttgatctg agccttggcc cctgacgaac ggcgggtggat ggaagatact 1560
gctctcaagt gctgaagcgg tagcttagct ccccgtttgc tgctgatcag tctttttcaa 1620
cacgtaaaaa gcgaggaggt tttgcaattt tgttggttgc aacgatctc cgttgatttt 1680
ggcctcttcc tccatgggag ggctgggagc atttgaagcg gttctctctt ctgcccgtta 1739

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<210> SEQ ID NO 157

<211> LENGTH: 1733

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 157, Example
157: designer Nial-promoter-controlled NADPH-dependent
chloroplast-targeted NADPH-dependent Butanol Dehydrogenase DNA
construct (1733 bp)

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<400> SEQUENCE: 157

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcccact tggagggtt 60
caaacgacct cgccgtacga acttttctgc gggggcgctc ccggatgta ggtgagcagt 120
gaccccgcgc gacttggaa ggttcaaacg accccgcccgt acgaactttt gtcggggggc 180
gctcccggat ggcccgcctc attgccaagt cctccgtctc cgcggccgtg gctcggccgg 240
cccgtccag cgtgcccctc atggccgcgc tgaagcccgc cgtcaaggct gcccccggtg 300
ctgcccgcgc tcaggccaac cagatgatga gatttacatt accaagagat atttattatg 360
gaaaaggatc actagaacaa ctaaaaaatc ttaaaggtaa aaaggcaatg ctagtacttg 420
gtggcggttc gatgaaaaga tttgatttgc tcgataaggt gttaggatc ttaaaggaa 480
ctggaataga agtaaaatta atagaaggag ttgaaccaga cccatcagtt gaaacagtgt 540

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tcaagggcgc	tgagttaatg	agacaatttg	aaccagattg	gattatagct	atgggtgggtg	600
gatcaccaat	tgatgctgca	aaagcaatgt	ggatttttta	tgaacacca	gaaaaaactt	660
ttgatgacat	taaagatccg	tttacagtac	cagaattaag	aaataaggct	aagttcctag	720
cgattccatc	aacaagtggg	acagcaacag	aagtaacagc	atcttctgta	attacagatt	780
ataagactga	aataaaatat	ccttttagctg	attttaatat	aactccagat	gtagctgtag	840
tagattcaga	attagctgaa	acaatgccac	ctaagttaac	tgcccataca	ggaatggatg	900
cattaactca	tgcaattgaa	gcttatgtag	caacattaca	ttcaccattt	actgatccac	960
tagctatgca	agcgattgaa	atgattaatg	aacatttatt	taaatcatat	gaaggcgata	1020
aagaagctag	agaacaaatg	cattatgctc	aatgttttagc	tggaatggct	ttctctaatag	1080
cactattagg	aatatgtcat	agtatggcgc	ataaacacag	ggctgtattc	catatccctc	1140
atggatgtgc	gaatgcaatc	tatttaccat	atgtaattaa	gtttaattca	aaaacttcat	1200
tagaaagata	tgctaaaata	gcaaaacaaa	tttcattagc	aggaaataca	aatgaggaat	1260
tagttgattc	attaataaac	ttagttaaag	aattaaataa	gaagatgcaa	ataccaacaa	1320
cattaaaga	atatggtatt	catgaacaag	aatttaagaa	taaggttgat	ttgatttcag	1380
aaagagctat	tggagatgct	tgtactggat	caaatccaag	acaattaat	aaagatgaaa	1440
tgaaaaagat	ttttgaatgc	gtatattatg	gtacagaagt	tgatttttaa	taaatggagg	1500
cgctcgttga	tctgagcctt	gccccctgac	gaacggcggg	ggatggaaga	tactgctctc	1560
aagtgctgaa	gcggtagctt	agctccccgt	ttcgtgctga	tcagtctttt	tcaaacgta	1620
aaaagcggag	gagttttgca	atcttgttgg	ttgtaacgat	cctccgttga	ttttggcctc	1680
ttctccatg	ggcgggctgg	gcgtatttga	agcggttctc	tcttctgccc	tta	1733

<210> SEQ ID NO 158

<211> LENGTH: 1745

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 158, Example 158: designer Nial-promoter-controlled chloroplast-targeted 3-Ketothiolase DNA construct (1745 bp)

<400> SEQUENCE: 158

agaaatctg	gcaccacacc	atggtagggt	gcgagtgacc	ccgcgcgact	tggaagggtt	60
caaacgaccc	cgccgtacga	acttttgcg	gggggcgctc	ccggatggta	gggtgcgagt	120
gaccccgccg	gacttggaa	ggttcaaacg	accccgccgt	acgaactttt	gtcggggggc	180
gctccccgat	ggccgcgctc	attgccaaat	cctccgctctc	cgcgcccgctg	gctcgcccg	240
cccgtcccag	cgtgcgcccc	atggccgcgc	tgaagcccgc	cgtaaggct	gcccccggtg	300
ctgccccggc	tcaggccaac	cagatgaccg	acatcgctcat	cgtcgccgca	gccccgaccc	360
ccgtgggcaa	gttcggcgcc	tcgctggcca	agatccccggc	gcctgagctg	ggcgctgccg	420
tgatcaaggc	cctgctggaa	aaaaccggcg	tgggcgcgca	ccagatcggt	gaagtcatca	480
tgggcccagg	gctggccggc	ggcgcggggc	agaaccggcg	tcgccaggcc	atgatgaagg	540
cgggcatcgc	caaggaaacg	ccggcgctga	ccatcaacgc	cgtgtgccc	tccggcctga	600
aggccgtgat	gctggcgccc	caagccatcg	cctggggcga	cagcgagatc	gtcatcgccg	660
gcggccagg	aaacatgtcc	gccagcccgc	acgtgctgca	aggcagccgc	gacggccagc	720
gcatgggcca	ctggaagatg	gtcgacacca	tgatcaacga	cgccctgtgg	gacgtgtaca	780

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acaagtacca catgggcatac acggccgaga acgtcgccaa ggcgcacgac atcaccctg 840
agcagcagga cgccttggcc ctggccagcc agcaaaaggc caccgcccgc caggaagccg 900
gcaagttaa ggacgagatc gttcccgtcg ccattccgca ggcgaagggc gatccggtga 960
tgttcgacac cgacgagttc atcaacaaga agaccaacgc cgaagcgtcg gccggcctgc 1020
gtccggcctt cgacaaggcc ggctcggtag cgcggggcaa cgctccggc atcaacgacg 1080
gcgcgcgcgc cgtgatggtc atgtcggcgc ccaaggccga gcaactgggc ctgaagccgc 1140
tggcgcgcgc cgcagcttc gccaccagcg gcttggaacc cgcaccatg gccatgggccc 1200
cggtgccggc cacgcgcaag gcgctggagc gcgcccgtcg gcaagtcggc gacgtggacc 1260
tgttcgagct gaacgaagcc ttcgcccgc aagcctgcgc ggtgaacaag gagctgggcg 1320
tggaccgggc caaggtcaac gtcaacggtg gcgccatcgc catcgccac ccctcggcg 1380
cctccggctg ccgctgtctg gtgacgtcgc tgcacgaaat gcagcgcgc gacgccaaga 1440
agggcgtggc cgcgctgtgc atcggtaggc gcatgggctg gtcgctggcc gtcgagcgt 1500
gataaatgga ggcgctcgt gatctgagcc ttgccccctg acgaacggcg gtggatgga 1560
gatactgctc tcaagtgtg aagcggtagc ttagctcccc gttcgtgct gatcagtctt 1620
tttcaacacg taaaagcggc agggattttg caattttgtt ggttgtaacg atcctccgtt 1680
gattttggcc tctttctcca tgggcgggct gggcgtattt gaagcggttc tctcttctgc 1740
cgtaa 1745

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<210> SEQ ID NO 159

<211> LENGTH: 1439

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 159, Example
159: designer Nial-promoter-controlled chloroplast-targeted
3-Hydroxyacyl-CoA dehydrogenase DNA construct (1439 bp)

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<400> SEQUENCE: 159

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggagggtt 60
caaacgaccc cgcctacga actttgtcg gggggcgctc ccgatggta ggtgagcgt 120
gaccccgccg cacttggaa ggttcaaac accccgcct acgaactttt gtcggggggc 180
gctcccggat ggcgcgcgc attgccaagt cctccgtctc cgcggccgtg gctcggccgg 240
cccgtccag cgtgcgccc atggccgcgc tgaagcccgc cgtcaaggct gcccccggtg 300
ctgcccggc tcaggccaac cagatggaga ttcggaagat cgcgctgac gccgcaggc 360
agatggggtc gggcatcgc caggtggcgc ccagtcggg tacgaggtc gtgctaatg 420
acgtcgagga gctgagcctc aagaaggggc tcgagccat ccgaaggtcg ctgaccgct 480
tcttcgcaa ggagaagatc acccaggagg aggcgaaaa ggccctggcc cgcataaga 540
cgacgctcaa ccccgcgcac ttcgaggact gcgacctggt cgtcgaggcc atcgtggaga 600
acgagtcggt caaggtaag ctcttcaga cgctcgaaa ggtggtgaa cctgaggccg 660
tcttcgcttc caacacctc tcgattccca tcaccaagct ggccagctac acctccgccc 720
ccgagcgttt catcggcatg cacttcatga acccgggtgc gctgatgaa ctcgtcgagg 780
tcatccgccc ctacaagacc tcggaagagg tcaccgggt ggtcatggcg acggccgaga 840
agatgggcaa ggtgcccgtc gaggtcaacg actaccgccc ctctctctcc aaccgctg 900

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tcatccccc  gctcaacgag  gccatccaag  cggtcacgga  gggcgtggcc  acccccgagg  960
ccatcgacac  cgtgatgaag  ctgggcatga  accaccccat  gggcccgtg  acgctcgccg  1020
acttcacg  cctcgacacc  gtgctggcca  tcatggaggt  gctgcacgag  ggctttggcg  1080
acagcaagta  ccgcccctcg  ccgctgctca  agaagatggt  ccaggcgggt  ctgctgggccc  1140
gcaagagcgg  gcagggggtc  tacaagtacg  acgagaaggg  gaacaagatc  ggctagtata  1200
tggaggcgct  cgttgatctg  agccttgccc  cctgacgaac  ggcggtggt  ggaagatact  1260
gctctcaagt  gctgaagcgg  tagcttagct  ccccgtttcg  tgctgatcag  tctttttcaa  1320
cacgtaaaaa  gcggaggagt  tttgcaattt  tgttggttgt  aacgatcctc  cgcttgattt  1380
ggcctctttc  tccatggcgg  ggctggcgct  atttgaagcg  gttctctctt  ctgcccgtta  1439

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<210> SEQ ID NO 160

<211> LENGTH: 1337

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 160, Example
160: designer Nial-promoter-controlled chloroplast-targeted Enoyl-
CoA Dehydratase DNA construct (1337 bp)

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<400> SEQUENCE: 160

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agaaaatctg  gcaccacacc  atggtagggt  gcgagtgacc  ccgcccact  tggaaagggt  60
caaacgaccc  cgccgtacga  acttttgtcg  gggggcgctc  ccggatggt  ggggtcgagt  120
gaccccgcgc  gacttgaag  ggttcaaacg  acccccgct  acgaactttt  gtcggggggc  180
gctcccggat  ggcccgcctc  attgccaagt  cctccgtctc  ccgcccgtg  gctcccgg  240
cccgtccag  cgtgcccacc  atggccgcgc  tgaagcccgc  cgtcaaggct  gcccctgg  300
ctgcccggc  tcaggccaac  cagatgacgg  ttcgactgga  atacgatggc  gggttcgcgc  360
acctgacgct  cagcccggc  caggtcctga  atgcccctc  tttcgagctg  ctgcccgg  420
tgagccgggc  gcttgcccgc  gtcgcccgaat  ccgatgccc  ccgcccctg  gtcacggggc  480
agggcgacaa  ggcgttctgc  gccggcggc  acattcccga  gctgatgaat  cggcccctc  540
tgcaagagct  cgaaggggccc  gcgaaaggcc  aggcgggtgt  cagcccgatc  gccgagctga  600
agattccgctc  tctgcccctc  atccagggtt  atgccttcgg  cggcccggctg  gagcttgc  660
tggcatgcac  attcccggct  gccactgatc  gcccggcct  ggggctgccc  gaggtcaagc  720
tcggcctgat  cccgggttat  ggccgaacgc  agcgtctgcc  gaggtgatc  ggcgaggggc  780
gcgcactcga  cctgatcatg  tccggcccga  cgatagacgg  cggggaagcc  gagcgaatcg  840
gcctggtcaa  tcgatagac  aacgagggga  cggcccctg  gatcggcaag  cggtttctg  900
agccttatct  caagcacagt  ctctgcccct  tgtattttgc  ccgcccggcc  gtgcagagg  960
gagggcgggt  cgccattgcg  gatggcctgc  gcacgagcgc  ggatctttcc  acgctggctt  1020
accggagcca  ggatgcggcc  gaggggctgc  ggccttttgc  ggaaaaacgg  cccgctctt  1080
tcaaggactg  ctgataaatg  gagggcctcg  ttgatctgag  ccttgcccc  tgacgaacgg  1140
cggtgatgg  aagataactgc  tctcaagtgc  tgaagcggta  gcttagctcc  ccgtttcgtg  1200
ctgatcagtc  tttttcaaca  cgtaaaaagc  ggaggagttt  tgcaattttg  ttggttgtaa  1260
cgatcctcgg  ttgattttgg  cctctttctc  catggggggg  ctgggctgat  ttgaagcgg  1320
tctctctctc  gccgtta  1337

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<210> SEQ ID NO 161
<211> LENGTH: 1736
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 161, Example
    161: designer Nial-promoter-controlled chloroplast-targeted
        2-Enoyl-CoA Reductase DNA construct (1736 bp)

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<400> SEQUENCE: 161

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt    60
caaacgaccc cgcctacga acttttctgc gggggcgctc ccggatggtg ggtgctgagt    120
gaccccgccg gacttgaag ggttcaaacg accccgcctg acgaactttt gtcggggggc    180
gtcccggatg ggccgcctc attgccaagt cctccgtctc cgcggccgtg gctcggcccg    240
cccgtccagc cgtgcgccc atggcgcgc tgaagcccgc cgtcaaggct gcccccgtgg    300
ctgcccgcgc tcaggccaac cagatggccg gcgcgcagca ggatcttgcc gctgcgtccg    360
ggcttctcgc tggccgcggc gcccttggcg gcatcgtgca ggtcgaacac cgettccacc    420
ggcagcgcca ggctgccatc gagcgcggcg gtgagcagtt ccgcgatcat gcggcgcttg    480
tcctcggcct tggtgccctg catcaccttg ctgcccaga agccacgcac ggtggcctgc    540
ttgaagatca catcgcctg ggatatctgc agcggctcgc cggtcatcga gccaaaaggaa    600
atcagctcgc cgccttcgce cagcaaggcc atcagctcac ccgctgcatt gccggccacc    660
gaatcgatgg cgcgcacgat gggcgcatcg ccggccagcg cgcgcacctt gtctcgccag    720
cctgcttgcg cagtggagat tgcgttgccg atgcccagcg ctttcagctc gtccacgccg    780
gcgtcgcggc gcaccagggt gatcacgttg atgcccgtg cggcggcgag catcgccacc    840
gtcttgccga ccgcaccgtt ggcggtgttc tgcacgatcc agtcgcccgt tttcacctgc    900
aggaattcga tcagcatcag cgcgctcagc ggcatggcga tcaactggca accacgctcg    960
tcgtccaggc catccggcaa cggcaccacg ccggaggcgt cggcaaggaa gtactcggcc   1020
caggcctcat gcacaccgcg ggcgaccacg cgtgggcaa cctgcaagcc ctcgacaccc   1080
tcaccagcgc catcgatgac acccgcctg ctgctgccc cgtggctgg cagttccggc   1140
ttgtagccgt aattgccgcg cacggtccac aggtcatggt tatggatcgg cgcgcgccgc   1200
atcgcaacgc gcacctggcc cttgctggc tgcggcgtgg ggcgctcgc cagttcgagc   1260
accttggcgc gatcgcgaa ttgggtatgg atggctgcgc gcatggagggt ctctgcggc   1320
gcacgctctt gctgcgacgc gcccgatcgt tgtgaaagggt ggcgcatgc tatcggcagg   1380
gctgcaagga agggatgaag cgaacggaac tgcgtgtgta agttgtggc gtgcgcgcgt   1440
agtgacgatg ctctgctgca gcgccggagg actgcgtgca ggcgcaccct cattaatgg   1500
aggcgctcgt tgatctgagc cttgccccct gacgaacggc ggtggatgga agatactgct   1560
ctcaagtgct gaagcggtag cttagctccc cgttctgctg tgatcagtct tttcaacac   1620
gtaaaaagcg gaggagtttt gcaattttgt tggttgtaac gatcctccgt tgattttggc   1680
ctctttctcc atggcgggcg tggcgctatt tgaagcgggt ctctctctcg ccgtta    1736

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<210> SEQ ID NO 162
<211> LENGTH: 2036
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 162, Example
    162: designer Nial-promoter-controlled chloroplast-targeted Acyl-

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CoA Reductase DNA construct (2036 bp)

<400> SEQUENCE: 162

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agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaagggtt    60
caaacgaccc cgcctacga acttttctcg gggggcgctc ccggatgta gggtcgagt    120
gaccccgccg gacttggaag ggttcaaacg accccgcctg acgaaacttt gtcggggggc    180
gctcccggat ggccgcctc attgccaagt cctccgtctc cgcggccgtg gctcggccgg    240
cccgtccag cgtgcgcccc atggcgcgc tgaagccgc cgtcaaggct gccccctgg    300
ctgccccggc tcaggccaac cagatggatg acgataatgt tgtaaaacc atttactatg    360
gtaaaataga ggttcaaat aatggcacgg gagtatggga gcctaccgtt gagggttga    420
atgagttttt aaaccggagt atggaactat cacagagtct tttacaacta ggctttagta    480
gaagggttag agtggtgagc gagatgggga agatttgag ggagaagctt tcgttagtcg    540
aggagaaact agctccta atttctaaga atacaggcta cagcgtggag aatgtgaaaa    600
tgagcttaag actggttgaa gaagtgttta atgagaccaa tattgttgag ctcttcgata    660
aaggcttaat cgggggatgg cgtagtcttg acaagccctg tgaatcatt gatggggagt    720
tcgtgtgaaa tagaccgcta ggcgatccc ttataatc ctcggaaac accgtcatal    780
cagctattct ccacagatg gtttcttag catcggggaa cgtaactata ctaaggccgt    840
ctttcagcaa ctaccaagca gtatcgaaa tttttaaacc acttttcgat ctagcagaca    900
gctccgtaga aggtgtaga gaaatggctt cggctcttct ggtcgcatal ttaaacatg    960
agagcaaggt atttgaacac ctattagcat cagcacctct cggcatcgtc aattactggg   1020
gccccggagc aggtagaagc gtgatcgcta gtagggtttt gaagaatccg tttcactcta   1080
agttaatcgt caatggacct ctaacggggt tagcgataat agatgaagag tcagcgtcgg   1140
aaaaagtagc ctacggatta gcgagggatg tggtagtcta tgatcaacag ttatgtagct   1200
ctcccactta cgccatattc ataggttcga aagatagcgc gttgaagttt gcacagagac   1260
taggggaagc cctgaataat gtggggagaa ggttcccccg tgatttgaag gaaggagaac   1320
tgtacaattt aatactgctt aggaaaaacc ttgagatcca aggtgtgaga gttttctact   1380
cggaaaaacc cggaaatgct tggacgattg cggtgaaaac actagagtca gtcactaatt   1440
ttgcatatag tttaaaatat ccacatacaa tccctaggag acggttcatt gaaataatag   1500
tgttgaaaga cgccaaagaa ctcaaggaga cgatcttaca cctaattgaa gacttgagga   1560
gaaacggggt tgataagttc cagacagcat cgataaagggt ttctgaaaga aaccttaacc   1620
acttattgaa ggttctctat attcttggga tttacagggt tgtccaata ggggaatcct   1680
tttttagaac gccgttagaa ccgtacgatg gtgaattctt acctaaatac ttcacttaca   1740
cgatgtatct tagatttacc gagaagtcgg atgcgctaaa acaccctgaa tgataaatgg   1800
aggcgtcctg tgatctgagc cttgccccct gacgaacggc ggtggatgga agatactgct   1860
ctcaagtgct gaagcggtag cttagctccc cgttctgtgc tgatcagtct tttcaacac   1920
gtaaaaagcg gaggagtttt gcaattttgt tggttgtaac gatcctccgt tgattttggc   1980
ctctttctcc atggggcggc tgggcgtatt tgaagcgggt ctctctctcg cgttta    2036

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<210> SEQ ID NO 163

<211> LENGTH: 1625

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 163, Example
    163: designer Nial-promoter-controlled chloroplast-targeted
    Hexanol Dehydrogenase DNA construct (1625 bp)

<400> SEQUENCE: 163
agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt    60
caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatgta gggtgcgagt    120
gaccccgcgc gacttgaag ggttcaaacg accccgcctg acgaactttt gtcggggggc    180
gtccccgat ggcgcgcctc attgccaagt cctccgtctc cgcggcctg gctcggcccg    240
cccgtccag cgtgcgcccc atggcgcgc tgaagccgc cgtcaaggct gccccctgg    300
ctgccccgc tcaggccaac cagatggaac tcgacctga cggccccgg gttggtgaag    360
tgctgatcaa gtacaccgc gcggggtgt gccattcga cctgcacttg accgacgggg    420
acctaccgc gcgctatcca atcgtcggg ggcacgagg gtcaggcatc atcgaggacg    480
tcggacctgg ggtcaccaag gtcaaacag cggatcacgt tgtttgcagc ttcattccga    540
actcgggaac ctgtcgtac tcgcccaccg gacgctcaa cctctcgat atgggcgcca    600
ccatcctga aggtgcatg cccgacgca gttaccggt ccacagtaac ggctggatt    660
tcggtgcat gtgatgctc ggcacattc ccgaacgcgc aactatctc cagcattcgg    720
tggtcaagat cgacgactg ctgccctcg agaccgcgt ggtcgtcggc tgcggcgtgc    780
cgactggctg ggcacctcc gtctatgcc gcggggttcg ttgcggtgac accaccgtca    840
tctatggcgt cggcggcctg ggagtcaacg ccgtccaagg cgcggtgagt gcgggcgcga    900
agtacatcgt ggtcgtcgt ccggttgcgt tcaaacgcga caccgcctc aagtccggcg    960
ccaccacgc gttegcgcac gccgccaccg ccgcgccaa ggtcgacgaa ctgacctggg   1020
gacaggggtc cgatcaggcg ctgatcctg tcggcacctg cgacgaggac gtggtctcgg   1080
cggcgactgc ggtgatcgtt aagggaggca ccgctcgtg caccggaactg gcggaccag   1140
caaaactcac ggtgcacgtt tcgggaacgg acctgacgct taacgagaag acaatcaagg   1200
gcacgttgtt cggctcgtcc aatccgcaat acgacatcgt acggctgctc cgtctctacg   1260
acgccggcca gctaaaactc gacgatctga tcaccaccg atacacgctc gaccagggtca   1320
accagggcta ccaggatctg cgagacgca agaacatccg cggcgtgatc atccacgcct   1380
gataaatgga ggcgctcgtt gatctgagcc ttgccccctg acgaacggcg gtggatggaa   1440
gatactgctc tcaagtctg aagcggtagc ttagctcccc gtttcgtgct gatcagtctt   1500
tttcaacacg taaaagcgg agggattttg caattttggt ggttgaacg atcctccggt   1560
gattttggcc tctttctcca tgggcgggct gggcgtatth gaagcgggtc tctctctcgc   1620
cgtaa                                             1625

<210> SEQ ID NO 164
<211> LENGTH: 1249
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 164, Example
    164: designer Nial-promoter-controlled chloroplast-targeted
    Octanol Dehydrogenase DNA construct (1249 bp)

<400> SEQUENCE: 164
agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcgcgact tggaaagggt    60

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caaacgaccc cgccgtacga acttttgtcg gggggcgctc ccggatggta gggtgccgagt 120
gaccccgcgc gacttggaag ggttcaaacg accccgcgct acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
cccgtccag cgtgcgcccc atggccgcgc tgaagccgcg cgtcaaggct gccccctgg 300
ctgccccgcg tcaggccaac cagatggttg gaggccaaga agccgctggt gattgaggac 360
attgaggtgg cgcacaccca ggcttggcag gttcgcacga agattacagc cactggcgtt 420
tgccacacgg attctttttc gttgagcggc tctgatcctg agggctctct tcccgtggtc 480
cttgcccatg agggcgcccg catcgtggag agcgttggcg agggcgtaac caactttaag 540
gcccggcgtc atgtcattgc cctctacata ccccagtcca atgagtgcaa attctgcaag 600
agcggcaaga caaatctctg ccagaagatt cgcctcaccg agggcgctgg tgtcatgccc 660
aatggatcct cccgcttctc gtgcaagggt cagcagctgt tccatttcat gggcacctca 720
actttcgccc agtacgcggt ggtggccgac atateggtga ccaaaatcaa cgagtcggct 780
ccattggaga aggtgtgctc tctgggctgt ggcatttcca cgggctatgg tgccgccttg 840
aacaccttta ggtggaacct ggcagcactt gcgccgctcg gggctctggg gctggtggac 900
tggcagtggt tctgggctgc aagaaggctg gcgcccga ggtctacggc atcgacatca 960
atccctccaa attcagctg gccaggaagt tcggcttccg cgaactttaa tggaggcgt 1020
cgttgatctg agccttgccc cctgacgaac ggcggtggt ggaagatact gctctcaagt 1080
gctgaagcgg tagcttagct ccccgtttcg tctgatcag tctttttcaa cacgtaaaaa 1140
gcccaggagt tttgcaattt gttggtgtt aacgatcctc cgttgatttt ggcccttttc 1200
tccatgggcg ggctgggctg atttgaagcg gttctctctt ctgcccgtta 1249

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<210> SEQ ID NO 165

<211> LENGTH: 1769

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- Sequence No. 165, Example
165: designer Nial-promoter-controlled chloroplast-targeted Short
Chain Alcohol Dehydrogenase DNA construct (1769 bp)

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<400> SEQUENCE: 165

```

agaaaatctg gcaccacacc atggtagggt gcgagtgacc ccgcccactt tggaaagggt 60
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gaccccgcgc gacttggaag ggttcaaacg accccgcgct acgaactttt gtcggggggc 180
gctcccggat ggccgcgctc attgccaagt cctccgtctc cgcggccgtg gctcggcccg 240
cccgtccag cgtgcgcccc atggccgcgc tgaagccgcg cgtcaaggct gccccctgg 300
ctgccccgcg tcaggccaac cagatgatca tcaaaccgcg cgtgcgtggc ttcactctgcg 360
tgaccactca tccggtcggc tgcgaggcca acgtcaagga acagatcgac tacgtgactt 420
cgcaacggccc gatcgccaac ggcccgaaga aggtgctcgt gatcggcgcg tcgaccggct 480
acggcctcgc ggcccggatc tcggccgctc tcggctcggg cgcggacacg cttggcgtgt 540
tcttcgagcg cgcggcagc gacaccaagc cgggcaccgc cggctggtac aacagcggc 600
cgttcgagaa attcgcgccg gaaaaggggc tctatgcgcg cagcatcaac ggcgacgcgt 660
tctccgacaa ggtcaagcag atcacgatcg acacgatcaa gcaggacctc ggcagggtcg 720
atctggtcgt ctacagcctg gccgcgcccg gccgcacgca tccgaagacg ggcgagacga 780

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tcagctcgac gctgaagccg gtcggcaagt cggtgacgtt ccgcgccctc gacaccgaca 840
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tcgcccgtgat gggcgccgag gactggcaga tgtggatcga cgcgctcgcc gacgccggcg 960
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acaaggaact cgatcccag gtgcaggcgc aggtcgtcgc gatgtgggac aaggtcacga 1380
acgacaacct gtacgagatg accgacttcg ccggttaca gaccgagttc ctgctctgt 1440
tcggcttca gatcgccgcg gtcgactacg acgcccagct gaaccggac gtgaagatcc 1500
ccggcatcat cgacacgagc gcctgataaa tggaggcget cgttgatctg agccttgccc 1560
cctgacgaac ggcggtggat ggaagatac gctctcaagt gctgaagcgg tagcttagct 1620
ccccgtttcg tgctgatcag tctttttcaa cacgtaaaaa gcgaggaggt tttgcaattt 1680
tgttggttgt aacgatcctc cgttgatttt ggcctcttc tccatgggcg ggctgggcgt 1740
attdgaagcg gttctctctt ctgccgta 1769

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<210> SEQ ID NO 166

<211> LENGTH: 6110

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 166, example
 166: a designer hox-promoter-controlled Formylmethanofuran
 dehydrogenase (Fmd) DNA construct (6110 bp)

<400> SEQUENCE: 166

```

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc 60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgagcgt ctataaaaaa gtgacatgtc ccgtctgcgg agggctctgt 240
gatgacatag aggttctcta tgatgggaaa acaataaaaa caaggaatgc ttgcaggatg 300
gggaatgcaa agtttcagga gatggtcagc tcccacagaa tacttagacc ccagattaaa 360
actgaaagtg gtttcagatc cgcagaatgg gatgaagccc ttgacgctgc agcagagata 420
cttacagagt caaaaagacc aaccctatc atgggtagtg agatgtcaac tgaagccatg 480
gctgcaggtc ttgaacttgg tgagtatcta aatgccatgg tgattcaaa tgcaaccata 540
tgtcatggcc ccacattaat gggaatccag gaagctggac agagcgtgc tacggcaggt 600
gagataaaga atagagctga cgtcatcatt tattgggta caaatgttat ggactccatg 660
cctcgccaca tgtcacgtta cagcatatc atgcgcgat tttcagaga acgtggtaaa 720
aaagatagaa ctgtcatctc cgtggatccc cgtgaaacag caacaactaa agcctccgat 780
atccatctgc agctgaaacc aaactcagat tatgaacttt ttcagcact gcttacagtt 840
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ataaaggaag	tcgcagacat	aatgctgaat	gcgcagtttg	gagccatcta	tgagagtctt	960
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gcacttaatg	aacacaccaa	atltacaata	ggagccataa	gaggtcattg	taatgttgca	1080
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gagtatatga	aagagattcc	tgtaatatgc	atagatattg	ccccatgtcc	aacaacactt	1320
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cgctttgaca	atgttcccat	ccaccataag	gcattcaca	catcaccatt	cctgaaaca	1440
gagagcaacg	aacatactct	tagacagatc	cttgagaggg	ttaaatcaat	taaaggcgaa	1500
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gagatctcaa	cattcaaaaga	agatggatcc	cttatgatcc	tattcaaagg	agatctatcc	2220
gaaaaaaatc	cggagggtaa	tctatacatc	aattataata	agaaccttca	cattctagag	2280
aatgagaccg	atgaagggag	agtcatcaca	aaaaagggaa	ttaaagtaat	ctacaacagt	2340
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atggctgtca	tcatcaacag	aaagggatg	cctggagaaa	cagtcacagg	tcttagaata	3000
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tcctggtatt	ttgttgatgt	gatcgttcca	gaaaaagaaa	agaagataat	tgaatctgt	3180
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aaaagataaa	tggaatata	tttaaaaaat	ggtattgtat	ttgatccagc	aaataatatt	3300
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tacactataa	aagatgggat	gatcatggtt	gatcatggaa	aaattgttaa	attagtagat	4800
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gtatataaaa	aaatagggga	gtaaatgtta	tgtgttaatc	aagatcattg	tttaggggtg	5460
ggagcatgty	ttatagtatg	tcctgttaat	caagaaatct	atccagaggt	tattggaggt	5520

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aatgggccag atacaacaga tgtggtaatg ttagttgaaa atggagtaat aaaactattc 5580
catccagaaa aatgtgtaac atgtatgaaa tgtaatgata catgtccaac aaaggcaata 5640
tatcataagg gagactaatg aagtaagtag gaagcagggg gcaggggaaa gaaaattgac 5700
aactgtacaa gattaatcgc gtctctgagc aatgaccaa tacatctacc tccacggttt 5760
tcttccagcc ccctatctgc gaaagcaca gatattagca agcgtttcgc ccaaattcac 5820
atacagctaa caatccctga tctcaatgct ggtgaatttt ctcagttaac aatcacgcgc 5880
caaatcaac aagttgccgc aattttccct gataattctg aaccaataac gctgataggt 5940
tctagtttag gcggttaaac tgctgcttat ctaggacagc gatatttaca agtacaacgc 6000
ttagttttat tagcgcagct ttggtttttt atcccattgg ttgccccaaa tgggtgaaga 6060
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<210> SEQ ID NO 167

<211> LENGTH: 1538

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 167, example
167: a designer hox-promoter-controlled Formyl transferase DNA
construct (1538 bp)

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<400> SEQUENCE: 167

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gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgtagaaga ggtcatggag tttgatctgg tatgctcaa ggttgccgcc 240
gtagttccct gcacttatct taattatgcc tggaacgta catgcggcct ttataccttc 300
cttcattgcc tcccttaca cctcttcggt gacaccgtct ataactatct catatacccc 360
gttgacgttt tcaggatatc cgctgccctc aacctcatcc ttgagtgtca cgcacatctt 420
ctcattgggt gaggcgttga ggaacttgta cttgttgat ccaacctttg aacctgaagc 480
caccacacca cggggaatg gtgttaccgt gccttcaact gcggctattg catccactgc 540
agcctgggcc gcaaggaggg ctgatgcctg actgtcacc atgatgaaga agtttccgcc 600
tgcaacccc tcctttatc caaatcgcct tcaatgagg aagtcgctg acattatcgg 660
gattgagtg attttcttc catcaattc aagttcctc tctagccat cccgaagaa 720
cttgagttg aatcccacat tgagcttctc gtcctcgtca tctgagagcat cgaatactgc 780
ggttggtggt gctgtaagta taccatccc tatccttca aggagttcat ggtccaggtt 840
cttcttgat ggggttcaga tcattattat gtatccgggc cttccatcgg gtgtctctc 900
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acctggtgcc tcggttcgag ctatctttgc aagtttttg gttgctgag ttacaaggac 1020
ccttgaaacc ttgataccga aggcctccgc aaatgtatcc tctatttcaa caccatttat 1080
ctccattgaa gtaagtagga agcagggagc aggggaaaga aaattgaca ctgtacaaga 1140
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ctatctcgca aagcacaaga tattagcaag cgtttcggcc aaattccat acagctaaca 1260
atcctgata tcaatgctgg tgaattttct cagttaacaa tcacgcgcca aattcaaca 1320
gttgccgcaa ttttccctga taattotgaa ccaataacgc tgataggttc tagtttaggc 1380

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ggtttaactg ctgcttatct aggacagcga tatttacaag tacaacgctt agttttatta 1440
gcgcaggttt ggttttttat cccattggtt gcccaaaatg ggtgaagaag ctgtcacaag 1500
ttggcaacaa acgatatagg ttctctcttc tgccgtta 1538

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<210> SEQ ID NO 168
<211> LENGTH: 1631
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 168, example
168: a designer hox-promoter-controlled 5,10-Methenyl-
tetrahydromethanopterin (H4 methanopterin) cyclohydrolase DNA
construct (1631 bp)

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<400> SEQUENCE: 168

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gtcaagcatt tgggatgatt tcccctcaca agttctctca attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgagtga aagtatgaac atagcagcaa agaaaattgc tgataaaatg 240
gttcaagatg cagaaaaatt aaaagtaatt ccatcaacat tatccaatgg aacatctatt 300
atagattgtg gtgtaaatgc taagggaagt attaaagggt gggaattatt tacaaaagta 360
tgtcttggty gaatttgtga tgttggaatt tcaatacctg gtgacttaag tgatataatg 420
gctatgcctg ctgtaagggt taaaactgat tttccagcat taaccactct tggttctcag 480
aaagcaggat ggaaaataga tgttaatgga tattatgcta taggttctgg acctgctcag 540
acacataaat ttaaagataa taacatztat aaaataacta actatattga agattcagat 600
attgcagtta ttactcttga agcagataaa ttacctgatg aagatattgc taattatatt 660
gcagatgagt gtgggtgtaaa accagaaaat ttaaccatac ttgttgacc tacatcttct 720
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gaaccaataa cgctgatagg ttctagttta ggcggtttaa ctgctgctta tctaggacag 1500
cgatatttac aagtacaacg cttagtttta ttagcgcag tttggttttt tatccattg 1560
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ttctgccgtt a 1631

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<210> SEQ ID NO 169
<211> LENGTH: 1475
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 169, example
    169, a designer hox-promoter-controlled 5,10-Methylene-H4-
    methanopterin dehydrogenase DNA construct (1475 bp)

<400> SEQUENCE: 169

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca    180
gaggtagata tgatggttgt aaaaattggt ataataaaat gtggtaacat cgggacctca    240
cctgtgctgg acctgttact tgatgagagg gcagacaggg caaacataga tgtctgtgtc    300
gtgggttcag gtgcaaaaat gaaccccgat gaaatcgaaa gggcagtacc aacctgctt    360
gaaatggaga gggactttgt tatattcata agcccaaacc ccggtgcacc tggccctgca    420
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gacccgatga taggggcaag aaggagttc ctggacccaa cagagatggc atccttcaac    600
tccgacgtta taaaggtcct tgcattcaca ggcgcataca gggttgtgca gaacacaatc    660
gatgcaatga ttgcagatgt tgaagccgga aaggcacctg aacttcctca ggtggttaata    720
gacacagata agcggttga ggcagcaggg tacaccaacc catatgcaaa ggccaaggcc    780
atggctgcat atgagatagc aaccaaggtg gctgacatag acgtcagggg ctgcttcatg    840
gtacaggacc ctgaccagta cataccaatc gttgcctcag cacacgaaat gctatctgca    900
gctgccaaac ttgcaattga agcaagggaa atcgaaaagg ccaacgacac cgtgctgaga    960
acaccacacg gtaaggaagg caaaacatt agcaagaagg atcttctggc caagccagaa    1020
tagtgaagta agtaggaagc agggagcagg ggaagaaaaa ttgacaactg tacaagatta    1080
atcgctcttc tgagcaatga ccaaatacat ctacctccac ggttttcttc cagcccccta    1140
tctgcgaaag cacaagatat tagcaagcgt ttcgccc aaa ttcacatata gctaacaatc    1200
cctgatctca atgctggatg attttctcag ttaacaatca cgcgccaaat tcaacaagtt    1260
gccgcaatth tccctgataa ttctgaacca ataacgctga taggttctag tttaggcggg    1320
ttaactgctg cttatctagg acagcgatat ttacaagtac aacgcttagt tttattagcg    1380
ccagtttggg tttttatccc attggttgcc caaaatgggt gaagaagctg tcacaagttg    1440
gcaacaaaacg atataggttc tctcttctgc cgtaa                                1475

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<210> SEQ ID NO 170
<211> LENGTH: 2594
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 170, example
    170: a designer hox-promoter-controlled methylenetetrahydrofolate
    reductase and/or methylene-H4-methanopterin reductase DNA
    construct (2594 bp)

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<400> SEQUENCE: 170

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc    60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat    120

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccaogcctca	180
gaggtagata tgctactcta ccttgggacg gggatacagc cggcttctct tgacgatggt	240
ggggatgata tcttcccaca tgacggccat gatatggacg cggctacac cctcgatgga	300
ctgcaggtgt ttaatctgtt ccacacagat ggcacccctc tcggccttgg ggtcggaggc	360
tttttccatg cggttgacga tttcgtcggg gacaatcatc cggccaccg attggtgcat	420
atatttggcg gcccgggcgg atttcagggg catgacgcg gcaataattt tggctctttt	480
atgcaggccg cgctcccga ccaggccat aaagcgttca aaacgctcca tatcaaaaat	540
gcactggggt tgaataaagt cggcgccggc attgatcttc ttctccaggc gcatgaccg	600
gaactcaaag gggctcgcaa aggggttggc ggcggcgcg atgaagaagc ggggttctctg	660
gcccttaate tcttaccgc aggcgaattt tttctcatcc cggaggtcct tcaogatccg	720
gatcagctgg agggaatcca cgtcatggac gtttttcgcc gtcggatggt taccaaaaga	780
ttgatggtcg ccggacaggc agaggacgtt ccgcatcccc aggctgtagg caccagggag	840
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cccggcctgg aggacgtgga cccagcggc gatactcgac aggcggacga tggccgtctg	960
gttgtccgtc aggttcatgg catcaacgta gtccttgagg agagcggcgt gcttcttgat	1020
ctctgtagga tcggcgtgct tcggcggctc aatctcacg ctgaccagaa attgccctg	1080
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gaagttgcgg aaaaagttag caaacggag atagcgatga cgcactccac aattgccatg	1260
gctgcagagt tgaataacct ggctggaata gacgatatcg tcctctcgga tccgtcattt	1320
gccggtgaat tcaactgtgt gaaggacttc gattacaatg aagtcacaa agcccacaaa	1380
gacaatcccc aaacgattat gccgaagatc agggagaagg ttaacgaact cgccaagaca	1440
gttccaaaac ccccgaagg cgccatacac tttgttcac cgaagacct aggcctgaag	1500
gtaacgactg acgacaggga agcagtagct gatgcagacc tgataatcac ctggctgcca	1560
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ggtatcgag ataaggtgga agttacatcc tatcaccocg gctcagttcc tgagaacaag	1740
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cttgaaaga aagcccgcgg acatgccttc aaacttctg cagaacttat cgggcccgtg	1860
tgcgacatgt gtgcagcact taccgctata acctacgcg gactgcttgt ataccgatg	1920
gccgttatga atattctcgg tgcacctgcc ggattcagcc agatgatggc gacggaatca	1980
ctggaacaga tcaccgccta tatgaagaag gtaggcataa agaacctga agagaacctt	2040
gacccgggtg tattccttgg aactgcagac tcaatgaact tcggacctat cgccgagatc	2100
cttcccacag ttcttaaatc acttgagaaa agggcaaaat aatgaagtaa gtaggaagca	2160
gggagcagg gaaagaaaat tgacaactgt acaagattaa tcgcgtctct gagcaatgac	2220
caataacatc tacctccacg gttttcttcc agccccctat ctgcgaaagc acaagatatt	2280
agcaagcgtt tcgcccacaa tcacatacag ctaacaatcc ctgatctcaa tgctggtgaa	2340
ttttctcagt taacaatcac gcgccaaatt caacaagttg ccgcaatttt cctgataat	2400

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tctgaaccaa taacgctgat aggttctagt ttaggcgggt taactgctgc ttatctagga	2460
cagcgatatt tacaagtaca acgcttagtt ttattagegc cagtttggtt ttttatccca	2520
ttggttgccc aaatggggtg aagaagctgt cacaagttgg caacaaacga tatagttct	2580
ctcttctgcc gtta	2594

<210> SEQ ID NO 171

<211> LENGTH: 2819

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 171, example

171: a designer hox-promoter-controlled

Methyltetrahydrofolate:corrinoid/iron-sulfur protein

methyltransferase DNA construct (2819 bp)

<400> SEQUENCE: 171

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gtcaagcatt tgggatgatt tccccacaca agttcctcaa attattctcc tataaacaat	120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccaagcctca	180
gaggtagata tgtcaggcct taatcttccc ggcccgggcg gcagatatat actccaggca	240
gtattcatcc tggccgtaca gggcgggtggc ggcccgcaac aggcccatta atggcatatc	300
caggggatca aggataaagg aatccatacc tttagtgagg cacataacca taaaggcccg	360
gttcagcagt cgcccttccc gcaggccata ggaaacgttg gaaaggccgc agatggtgtg	420
aaacccgggg tatttttggc gcaggggcgc aaccgattcc agagcctcca cgcggtactg	480
gctgttaact cccagggggt taatcagggg atcaaggtag atatcgtctt ccggtatgcc	540
ggcctctacc agcccgggta ccagcttacc agcaacgcga aggcggctct ctaccgaatc	600
cggcatgccg ccgtcatcca tgcaaaagggc gataacctta gccttatatt tctgactaa	660
aggcaaaact tcctcccage gctgcttctc ggcgggtgat gagtttacca ttgctggcc	720
cttatgggct gccaggcccg cagccagggc ctccggcctg gggctatcaa tacacagggg	780
aatcaaacg acttcttgga cgatattcac gagccaggtc attaccttat tttcttcgcc	840
gatgcttgtc ccgcagttaa catcgataat atcggctccg gcctcggcct gcttctggc	900
cagttctctg atataatcct tatcccgtt agcaatagcc tgggcaatgg ctttgcggct	960
ggagttaatt aactctccga caacgagcat ttgcaggtaa ctgccatgga tgtataccga	1020
ttacttccaa aaacaaactg tggtaaatgt gacgagtcac catgcatggc cttcgccaca	1080
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cagaagctcg aggacctcct tgcgcccgtc gtgagggaaa tcaccttcgg cccagaaaag	1200
aaccaggtgg tggttggggg tgatgaggtc ctctacaggt tgaactcac atactataac	1260
cccacagccc tcggtgttga tcttccagat gacctacat cagaggagat caggaagagg	1320
gccagtgata tcatgaatct taaattcgag cgtaccggtg aggaactgac cctggatgcc	1380
atagcgtca ggaacaaatc aggaagccct gaaaaatttg cagaggcagc agggacctg	1440
gctgaaacta acttccctgt tgttctatgc acattcgatc cagaggcaat gagggctgcc	1500
ctcagggtec tgggggatca gagggcccctc atgtatgcgg ctacaaagga caacctccag	1560
gagatggctg atctttccat ttcatacggc tgccccctgg tgctcttctc accgggggac	1620
cttgaggaga tgaagaacct cacaagaaga ctgcggggcaa tgggccttac tgaattatc	1680

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ctggaccccc gaacattcac aggtgagggg ataggcgaca ccatagacaa ctttgcctatg 1740
ataaggcgcc ttgcagttga ggaaagggat gaggacttcc gcttccccat catgggcata 1800
ccagcactct caaggtttc agggagggat ccagttgagg ataacataaa ggaggccact 1860
gttgagccca cactcatgaa ccgctacgcc gacattctta tactcggagg aacagatatac 1920
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cagactgttg atccgggaat atatgagttt ggagacgttg atgagaactc ccctgtgata 2040
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gttacggcat atctccttgt gcttgacaca gagggaaggg ctgtggacgt tteactggct 2160
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ccacggtttt cttccagccc cctatctcgc aaagcacaag atattagcaa gcgtttcgcc 2520
caaattcaca tacagctaac aatccctgat ctcaatgctg gtgaattttc tcagttaaca 2580
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ctgatagggt ctagtttagg cggtttaact gctgcttacc taggacagcg atatttaca 2700
gtacaacgct tagttttat agcgcagtt tggtttttta tcccattggt tgcccaaat 2760
gggtgaagaa gctgtcaca gttggcaaca aacgatatag gttctctct ctgccgtta 2819

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<210> SEQ ID NO 172

<211> LENGTH: 2771

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 172, example
172: a designer hox-promoter-controlled Corrino iron-sulfur
protein DNA construct (2771 bp)

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<400> SEQUENCE: 172

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatggccgt ccagatttta cgtgatcgta gccgagctgc cgtccagaaa 240
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ggtatggaag tgcaggatat cgtacccgac tggcccagc ttctcaaaga tcccttcacc 420
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tgcgtagcta ctgttaaaga ggtcctgcag gccgtggggg taccctgggt agtggtaggt 600
tgcggcgatg tggaaaagga ccatgaggtc ctggaagcag tagccgaggc tgctgccggc 660
gagaatctcc tctgggtaa cgctgaacag gaaaactata aatccctaac ggcagcctgc 720
atggtccaca agcataatat catcggccgt tcgcccctgg atattaacat ttgtaaaaa 780
ctcaacatcc tgatcaatga aatgaacctg ccctggatc atatcgtcat cgaccgtcc 840

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atcggcggcc tgggttatgg tattgaatac tccttctcga ttatggaacg catccgtctg   900
ggggccctgc agggagataa gatgtctctc atgccgggtca tctgcaccgt aggctatgag   960
gcctggcgcg ccaaggaagc ctcggcaccc gtgagcgaat acccgggctg gggtaaggaa   1020
accgagcgtg gcatcctctg ggaagccgtt accgccactg ccctgctcca ggcggcgccc   1080
cacatcctcc tcatgcgcca tccggaagcc gtagccaggg tgaaggagaa tatcgaccag   1140
ttaatggtga gcaacgccta ttaaatggat aaattaacgg aacttctgaa attactctag   1200
aacacggaat ccatcgaaat aatgagttc aggattgatg ttgacgaact tgaactctac   1260
ctgatgcctg tggttcagca ggccatccag aaaacagttg aggttaggga ggcagttgag   1320
gcactccctg aggaggaatt tgaaccaccg gtaaagacat accctggtga ggttgcccag   1380
gtgaaactgg gtgagggtag aaggaacct gtttaccttg gcggtcagaa ggcctctac   1440
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atgccgggtc tcccaggccc gataagggag cacttcagcg acgtcatgga ggaccctggc   1560
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gcagaccttc agatgccaat gtctctgga acaaccaacg cgtgggggttc aaggagggcc   2100
tggatgaaga aggatgaatg gggacccaca gactacaggg gccctctatg ggagatagta   2160
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gtgaggcttc tgccggagat aggggaaacc ttcacaaggg aatacatgac gaccgaaaca   2280
cctgatctcc ggaatggat aactgaactt gaatattgat gaagtaagta ggaagcaggg   2340
agcaggggaa agaaaattga caactgtaca agattaatcg cgtctctgag caatgaccaa   2400
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aagcgtttcg cccaaattca catacagcta acaatccctg atctcaatgc tggtaattt   2520
tctcagttaa caatcacgcy ccaaattcaa caagttgccg caattttccc tgataattct   2580
gaaccaataa cgctgatagg ttctagtta ggcggtttaa ctgctgctta tctaggacag   2640
cgatatttac aagtacaacg cttagtttta ttagcggcag tttggttttt tatcccattg   2700
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ttctgccgtt a                                     2771

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<210> SEQ ID NO 173

<211> LENGTH: 7061

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 173, example
 173: a designer hox-promoter-controlled CO dehydrogenase /acetyl-
 CoA synthase DNA construct (7061 bp)

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<400> SEQUENCE: 173

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
gaggtagata tgtcacataa tgggatccat ggtcaaggcc ggtgtccct tttcctccaa      240
gtagggcaag atttcatcca cgggtgtacc gatggtctca tcagctatth tatcgataaa      300
gtcctctccc aggccctcct caacactacy acgtacaaag tcgtcgtgga ggaatcctt      360
cagagatttg ggcacccaga ccatccgggc gataccacca tcggcggaaa taaacttttt      420
gctgacgata taggtgcggc ccatgcccac aaagcccggg gctcgggtgc cacgcgcgat      480
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caggacaggc cttaacctgc ctccgttgcg gtttaaaata ggtggtaaat cccccagttt     2160
ttgacctct tccccgctga aacagcgaat aaccggcagg taataggctg tatcaggata     2220
accgacggga tggctcggac cataggtccg gatggcctgg tttaaagga tttccgcta     2280

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actggtagct gtaatggcgc cgtggtaaac ctcccgaac agggctaccg gctctttacc	2340
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gttcgggata tcgccagat agttgocctg gatctcccta agggatgata ggaocgcccc	3060
ctcgttgaac tgggattcgg tacaatagac aacaccaaac ccgtgatact ctgcataggc	3120
cacaacgtcc tccctggagc ggatatagtg gactacgtca ccgagaggga actcgaagac	3180
gaagttgagg tatgcccgat atgctgtgca gcaatagatg ttacaaggta cagcagggct	3240
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cagaaggcgg cggccatagc aaccggtgtg aaccggtggg gaatacccg cgtcctggga	4320
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ctctatgcag cggagaaccg ggaggaagca acggtgatgg tggccaaact ctgcataagg	4500
ccaacagaca cacccaaggg tcgccagatg aaactcagca actacataga cctccacaaa	4560

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aaatactttg gcacaattcc agatgacatc gacaggttca taaggacaga gaaggacatc	4620
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aagttcccaa aggaaccctc actccttgag aggtgaatgt ttgaagacat acccgttgat	4740
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aaggtcattt aacagcgacc ctaccaccat gagtggctctt tcagactttt ttatggccga	6540
tgcaaccatt tcagggggca tgacgggtgc attccttatt gatgttagag ctgggttcca	6600
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agattaatcg cgtctctgag caatgaccaa atacatctac ctcccaggtt ttcttccagc	6720
cccctatctg cgaaagcaca agatattagc aagcgtttcg cccaaattca catacagcta	6780
acaatccctg atctcaatgc tggatgaattt tctcagttaa caatcacgcg ccaaattcaa	6840
caagttgccg caattttccc tgataattct gaaccaataa cgctgatagg ttctagtta	6900

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ggcggtttaa ctgctgctta tctaggacag cgatatttac aagtacaacg cttagtttta 6960
ttagcgccag tttggttttt tatcccattg gttgccaaa atgggtgaag aagctgtcac 7020
aagttggcaa caaacgatat aggttctctc ttctgcccgtt a 7061

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<210> SEQ ID NO 174

<211> LENGTH: 1847

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 174, example
174 of a designer hox-promoter-controlled Thiolase (07) DNA
construct (1847 bp)

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<400> SEQUENCE: 174

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agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc 60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgggcaa agaaagtagt tttagctgtg catgtcgtac agccatcgga 240
acaatgggtg gatctcttag cacaattcct gcagtagatt taggtgctat cgttatcaaa 300
gaggtcttta accgcgcagg tgtaaacct gaagatgttg atcacgtata catgggatgc 360
gttattcagg caggacaggg acagaacggt gctcgtcagg cttctatcaa ggctggtcct 420
cctgtagaag tacctgcagt tacaactaac gttgtatgtg gttcaggtct taactgtgtt 480
aaccaggcag ctcagatgat catggctgga gatgctgata tcgttgttgc cgggtgtatg 540
gaaaacatgt cacttgcacc atttgcactt cctaattggcc gttacggata tcgtatgatg 600
tggccaagcc agagccaggg tggctctgta gacactatgg ttaaggatgc tctttgggat 660
gctttcaatg attatcatat gatccagaca gcagacaaca tctgcacaga gtggggctct 720
acacgtgaag agctcagatg gtttcagct aagagccaga acaaggcttg tgcagcaatc 780
gaagctggcg cattcaagga tgagatcgtt cctgtagaga tcaagaagaa gaaagagaca 840
gttatcttcg atacagatga aggcccaaga caggggtgta cacctgaatc tctttcaaag 900
cttcgtccta tcaacaagga tggattcgtt acagctggtg acgcttcagg tatcaacgac 960
ggtgctgcag cactcgtagt tatgtctgaa gagaaggcta aggagctcgg cgtaaacct 1020
atggctacat tcgtagctgg agcacttgct ggtgtctgct ctgaagtat gggtatcggg 1080
cctgtagcag ctactcagaa ggctatgaag aaggctggtg tcgagaacgt atctgagttc 1140
gatatcatcg aggctaacga agcattcgca gctcagctcg tagcagttgg taaggatcct 1200
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gagaagtacy aataatgaag taagtaggaa gcaggggagca ggggaaagaa aattgacaac 1440
tgtacaagat taatcgctgc tctgagcaat gaccaaatc atctacctcc acggttttct 1500
tccagcccc tatctgcgaa agcacaagat attagcaagc gtttcgcccc aattcacata 1560
cagctaacaa tccctgatct caatgctggt gaattttctc agttaacaat cacgcgcca 1620
attcaacaag ttgccgcaat tttccctgat aattctgaac caataacgct gataggttct 1680
agtttaggcy gtttaactgc tgcttatcta ggacagcgat atttacaagt acaacgctta 1740

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gttttattag cgccagtttg gttttttatc ccattggttg cccaaaatgg gtgaagaagc 1800
tgtcacaagt tggcaacaaa cgatataggt tctctcttct gccgtta 1847
```

```
<210> SEQ ID NO 175
<211> LENGTH: 1514
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 175, example
175: a designer hox-promoter-controlled 3-Hydroxybutyryl-CoA
dehydrogenase DNA construct (1514 bp)
```

```
<400> SEQUENCE: 175
agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc 60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgcaaaa gatttgtgta ataggtgctg gaacaatggg ctcaggcctc 240
gctcaagtat ttgcacaaaa tggctttgaa gtaattttac gcgatattga tatgaagttc 300
gtagaaaaag gattttggcacc aattgaaaaa atttacaag aaatggtgac aaagggaaaa 360
ttacagcaga tgagaaaaacg aattttaagc agaatcagag gtacaacaaa tttggaagac 420
gcaaaaagag cagattttgt agttgaagcg gctatagaaa atatggatct caagaaacaa 480
atattcaaaag agctagatga aatatgcaaa atggaaacaa tccttgctgc aaatacatca 540
tcactatcca taacagaaat agcaagtgcg acaaaaagac ctgagaaagt cataggatg 600
catttcttca acccagttcc agtaatgaaa cttgttgaag tcataaaagg attaaagaca 660
tcagagcaaa catttaaatgt cgtcagagaa ttggctttaa aagtagacaa aacacctata 720
gaggtcaaaag aagcacctgg atttgttga aataggattt taatcccaat gattaatgaa 780
gcaattggaa tacttgcagt ggtgttgca actgacaaga gcatagatga agctatgaaa 840
cttggtgcaa atcatccaat aggaccttgg gcattgtcta gtttgatagg caatgacgtc 900
gttcttgcta taatgaatgt gctttatgaa gagtacggcg attcgaata cagaccacat 960
ccacttctaa aaaaagtggg aagaggcgga ttgctgggta gaaaaactgg caaaggtttc 1020
tttgaataca aaattaatct ttaaggagg agaatatcat gatgaagtaa gtaggaagca 1080
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caaatatcct tacctccacg gttttcttcc agccccctat ctgcgaaagc acaagatatt 1200
agcaagcgtt tcgccccaat tcacatacag ctaacaatcc ctgatctcaa tgctggtgaa 1260
ttttctcagt taacaatcac gcgccaaatt caacaagttg ccgcaatttt ccctgataat 1320
tctgaaccaa taacgctgat aggttctagt ttaggcggtt taactgctgc ttaatctagga 1380
cagcgatatt tacaagtaca acgcttagtt ttattagcgc cagtttggtt ttttatccca 1440
ttggttgccc aaaatgggtg aagaagctgt cacaagttgg caacaaacga tatagttct 1500
ctctctctgcc gttta 1514
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<210> SEQ ID NO 176
<211> LENGTH: 1430
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 176, example
176: a designer hox-promoter-controlled Crotonase DNA construct
(1430 bp)
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<400> SEQUENCE: 176

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agaaaatctg gcaccacacc gcagaaat at aggggctagg agttgagggt actctggttc    60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat    120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca    180
gaggtagata tgatggaatt aaaaaatggt attcttgaaa aagaagggca tttagctatt    240
gttacaatca atagacaaa ggcatataat gcattgaatt cagaacact aaaagattta    300
aatgttggtt tagatgattt agaagcagac aacaatgtgt atgcagtat agttactggt    360
gctggtgaga aatcttttgt tgctggagca gatatttcag aatgaaaga tcttaatgaa    420
gaacaaggta aagaatttgg tatttttagga aataatgtct tcagaagatt agaaaaattg    480
gataagccag ttatcgcagc tatatcagga tttgctcttg gtggtggatg tgaacttgct    540
atgtcatgty acataagaat agcttcagtt aaagctaaat ttggtcaacc agaagcagga    600
cttgaataa ctccaggatt tgggtggaact caaagattag caagaatagt tggaccagga    660
aaagctaaag aattaattta tacttgtagc cttataaatg cagaagaagc ttatagaata    720
ggcttagtta ataaagtagt tgaattagaa aaattgatgg aagaagcaaa agcaatggct    780
aacaagattg cagctaagtc tccaaaagca gttgcatatt gtaaagatgc tatagacaga    840
ggaaatgcaag ttgatataga tgcagctata ttaatagaag cagaagactt tgggaagtgc    900
tttgaacagc aagatcaaac agaaggaatg actgcgttct tagaagaagc agcagaaaag    960
aattttcaaa ataaataatg aagtaagtag gaagcagggg gcaggggaaa gaaaattgac   1020
aactgtacaa gattaatcgc gtctctgagc aatgacaaa tacatctacc tccacggttt   1080
tcttccagcc ccctatctgc gaaagcacia gatattagca agcgtttcgc ccaaatcac   1140
atacagctaa caatccctga tctcaatgct ggtgaatttt ctcagttaac aatcacgcgc   1200
caaatccaac aagttgccgc aattttccct gataattctg aaccaataac gctgataggt   1260
tctagttagc gcggtttaac tgctgcttat ctaggacagc gatatttaca agtacaacgc   1320
ttagttttat tagcgcagct ttgggttttt atcccattgg ttgcccacaa tgggtgaaga   1380
agctgtcaca agttggcaac aaacgatata ggttctctct tctgccgtta    1430
```

<210> SEQ ID NO 177

<211> LENGTH: 1784

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 177, example
177: designer hox-promoter-controlled Butyryl-CoA dehydrogenase
DNA construct (1784 bp)

<400> SEQUENCE: 177

```
agaaaatctg gcaccacacc gcagaaat at aggggctagg agttgagggt actctggttc    60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat    120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca    180
gaggtagata tgatggaatt ccaatgaact agagaacaac aattagtaca acaaatgggt    240
agagaattcg cagtaaatga agttaagcca atagctgctg aaatcgacga aacagaaaga    300
ttccctatgg aaaacgttga aaaaatggct aagcttaaaa tgatgggtat cccattttct    360
aaagaatttg gtggagcagg cggagatggt ctttcatata taatagctgt ggaagaatta    420
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tcaaaagt	ttt	gtggtactac	aggagttatt	ctttcagcgc	atacatcatt	atgtgcatca	480
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ggtaaaaaga	tcggtgcttt	cggattaact	gaaccaggtg	ctggtacaga	tgctgcagga		600
caacaaacaa	ctgctgtatt	agaaggggat	cattatgtat	taaatgggtc	aaaaatcttc		660
ataacaaatg	gtggagttgc	tgaaaacttc	ataatatttg	ctatgacaga	taagagtcaa		720
ggaacaaaaag	gaatttctgc	attcatagta	gaaaagtcac	tcccaggatt	ctcaatagga		780
aaattagaaa	ataagatggg	gatcagagca	tcttcaacta	ctgagttagt	tatggaaaac		840
tgcatagtac	caaaagaaaa	cctacttagc	aaagaaggta	agggatttgg	tatagcaatg		900
aaaactcttg	atggaggaag	aattgggata	gctgctcaag	ctttaggtat	tgcaagaagga		960
gcttttgaag	aagctgttaa	ctatatgaaa	gaaagaaaac	aatttggtaa	accattatca		1020
gcattccaag	gattacaatg	gtatatagct	gaaatggatg	ttaaaatcca	agctgctaaa		1080
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atctttggty	gatatgggta	cactaaagaa	taccagtag	aaagaatgat	gagagatgct		1260
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attttaagat	agtgaagtaa	gtaggaagca	gggagcaggg	gaaagaaaat	tgacaactgt		1380
acaagattaa	tcgctctctc	gagcaatgac	caaatacatc	tacctccacg	gttttcttcc		1440
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ctaacaatcc	ctgatctcaa	tgctgggtaa	ttttctcagt	taacaatcac	gcgccaaatt		1560
caacaagttg	ccgcaatfff	ccctgataat	tctgaaccaa	taacgctgat	aggttctagt		1620
ttaggcgggt	taactgctgc	ttatctagga	cagcgatatt	tacaagtaca	acgcttagtt		1680
ttattagcgc	cagtttgggt	ttttatccca	ttggttgccc	aaaatgggtg	aagaagctgt		1740
cacaagttgg	caacaaacga	tataggttct	ctcttctgcc	gta			1784

<210> SEQ ID NO 178

<211> LENGTH: 2051

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 178, example 178, a designer hox-promoter-controlled Butyraldehyde dehydrogenase DNA construct (2051 bp)

<400> SEQUENCE: 178

agaaaatctg	gcaccacacc	gcagaaat	at	aggggctagg	agttgagggt	actctggttc	60
gtcaagcatt	tgggatgatt	tcccctcaca	agttcctcaa	attattctcc	tataaacaat		120
agatataagg	tcaaaacttg	agttatgagt	gctgagtaaa	aaattactct	ccacgcctca		180
gaggtagata	tgatgattaa	agacacgcta	gtttctataa	caaaagattt	aaaattaaaa		240
acaaatgtyt	aaaatgccaa	tctaaagaac	tacaaggatg	attcttcatg	tttcggagtt		300
ttcgaaaatg	ttgaaaatgc	tataagcaat	gccgtacacg	cacaaaagat	attatccctt		360
cattatacaa	aagaacaaag	agaaaaaatc	ataactgaga	taagaaaggc	cgcattagaa		420
aataaagaga	ttctagctac	aatgattctt	gaagaaacac	atatgggaag	atatgaagat		480
aaaatattaa	agcatgaatt	agtagctaaa	tacactcctg	ggacagaaga	tttaactact		540
actgcttggt	caggagataa	cgggcttaca	gtttagataa	tgtctccata	tgccgttata		600

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ggtgcaataa ctcccttctac gaatccaact gaaactgtaa tatgtaatag tataggcatg 660
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tttgcgtcgc aaatgataaa taaagctatt atttcatgtg gtggctcctga gaatttagta 780
acaactataa aaaatccaac tatggactct ctagatgcaa ttattaagca cccttcaata 840
aaactacttt gcggaactgg agggccagga atggtaaaaa ccctcttaaa ttctggtaag 900
aaagctatag gtgctgggtc tggaaatcca ccagttattg tagatgatac tgctgatata 960
gaaaaggctg gtaagagtat cattgaaggc tgttcttttg ataataattt accttgtatt 1020
gcagaaaaag aagtatttgt ttttgagaac gttgcagatg atttaatatc taacatgcta 1080
aaaaataatg ctgtaattat aaatgaagat caagtatcaa agttaataga tttagtatta 1140
caaaaaata atgaaactca agaatactct ataaataaga aatgggtcgg aaaagatgca 1200
aaattattct tagatgaaat agatgttgag tctccttcaa gtgttaaatg cataatctgc 1260
gaagtaagtg caaggcatcc atttgttatg acagaactca tgatgccaat attaccaatt 1320
gtaagagtta aagatataga tgaagctatt gaatatgcaa aaatagcaga acaaaataga 1380
aaacatagtg cctatattta ttcaaaaaat atagacaacc taaatagggt tgaagagaa 1440
atcgatacta ctatctttgt aaagaatgct aaatcttttg ccggtgttgg ttatgaagca 1500
gaaggcttta caactttcac tattgctgga tccactggtg aaggaataac ttctgcaaga 1560
aattttaca gacaaagaag atgtgtactc gccggttaat gaagtaagta ggaagcaggg 1620
agcaggggaa agaaaattga caactgtaca agattaatcg cgtctctgag caatgaccaa 1680
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aagcgtttcg cccaaattca catacagcta acaatccctg atctcaatgc tggatgaattt 1800
tctcagttaa caatcacgcg ccaaattcaa caagttgccg caattttccc tgataattct 1860
gaaccaataa cgctgatagg ttctagtta ggcggtttaa ctgctgctta tctaggacag 1920
cgatatttac aagtacaacg cttagtttta ttagcgcag tttggttttt tatcccattg 1980
gttgccaaa atgggtgaag aagctgtcac aagttggcaa caaacgatat aggttctctc 2040
ttctgccgtt a 2051

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<210> SEQ ID NO 179

<211> LENGTH: 1808

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 179, example
179: a designer hox-promoter-controlled NADH-dependent Butanol
dehydrogenase DNA construct (1808 bp)

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<400> SEQUENCE: 179

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agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc 60
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<210> SEQ ID NO 180

<211> LENGTH: 10538

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 180, example
180: a designer hox-promoter-controlled Energy converting
hydrogenase (Ech) DNA construct (10538 bp)

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<400> SEQUENCE: 180

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<210> SEQ ID NO 181

<211> LENGTH: 3416

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 181, example
181: a designer hox-promoter-controlled [NiFe]-hydrogenase MvhADG
DNA construct (3416 bp)

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<400> SEQUENCE: 181

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<210> SEQ ID NO 182
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 182, example
      182: a designer hox-promoter-controlled Heterodisulfide reductases
      (HdrABC, HdrDE) DNA construct (6695 bp)

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<210> SEQ ID NO 183

<211> LENGTH: 3407

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 183, example
183: a designer hox-promoter-controlled Coenzyme F420-reducing
hydrogenase (Frh) DNA construct (3407 bp)

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<210> SEQ ID NO 184

<211> LENGTH: 5417

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 184, example
184: a designer hox-promoter-controlled Methyl-H4MPT: coenzyme M
methyltransferase (MtrA-H) DNA construct (5417 bp)

<400> SEQUENCE: 184

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<210> SEQ ID NO 185

<211> LENGTH: 5042

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 185, example
185: a designer hox-promoter-controlled Methyl-coenzyme M
reductase (Mcr) DNA construct (5042 bp)

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<400> SEQUENCE: 185

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<210> SEQ ID NO 186

<211> LENGTH: 5450

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 186, example
 186: a designer hox-promoter-controlled Formate dehydrogenase DNA
 construct (5450 bp)

<400> SEQUENCE: 186

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ttagttttat tagcgccagt ttggtttttt atcccattgg ttgccccaaa tgggtgaaga 5400
agctgtcaca agttggcaac aaacgatata gggtctctct tctgccgta 5450

<210> SEQ ID NO 187

<211> LENGTH: 2324

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 187, example
187: a designer hox-promoter-controlled 10-Formyl-H4 folate
synthetase DNA construct (2324 bp)

<400> SEQUENCE: 187

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgttgtccaa ggtacccagt gatattgaga ttgcccaggc agccaaaatg 240
aaaccggtca tggaaactgc ccggggactg ggcacccaag aggacgaggt cgagctttat 300
ggtaagtaca aggccaagat ctcccctgat gtctatcgtc gcctcaaaga caagcctgac 360
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cgggagccct ccctgggacc cagctttggt atcaaaggcg gtgccgcccg cggtggttat 540
gcccaggtag taacctgga agatatcaac ctgcacttca ccggcgatat ccacgcccgtc 600
acctatgccc acaacctgct ggcggccatg gtggataacc acctgcagca gggtaacgtc 660
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tatgaatccc tgggctacgg caacctgccg gtggtcatgg ccaagaccca atactccttt 1680
tccgatgaca tgaccaagct cgggcccggc cggaacttta ccatcacctg gcgagaggtg 1740

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ttaggcggtt taactgctgc ttatctagga cagcgatatt tacaagtaca acgcttagtt 2220
ttattagcgc cagtttggtt ttttatccca ttggttgccc aaaatgggtg aagaagctgt 2280
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<210> SEQ ID NO 188

<211> LENGTH: 1487

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 188, example
188: a designer hox-promoter-controlled 10-Methenyl-H4 folate
cyclohydrolase DNA construct (1487 bp)

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<400> SEQUENCE: 188

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgctagcgcc gggccgcctc gactgtatcc ttcaagagca tggcaatggt 240
catgggacct accccgccgg gtacgggagt gatccaaccg gccttctggg ccgcactctc 300
gaagtggacg tcgcccacca gctttttctc gccgaccggg ttgataccta cgtcgataac 360
taccgcgccc tctttaatca tatccccggg aatcaactcc ggcttcccta cggccgcaat 420
gagaatgtcg gcctgcgggc attcggctgc caggtccctg gtgcgggagt gacagatggt 480
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cagctaaaca tccctgatct caatgctggt gaattttctc agttaacaat caccgcacca 1260
attcaacaag ttgccgcaat tttccctgat aattctgaac caataacgct gataggttct 1320
agtttaggcg gtttaactgc tgcttatcta ggacagcgat atttacaagt acaacgctta 1380

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gttttattag cgccagtttg gttttttatc ccattggttg cccaaaatgg gtgaagaagc 1440
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<210> SEQ ID NO 189
<211> LENGTH: 1487
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 189, example
189: a designer hox-promoter-controlled 10-Methylene-H4 folate
dehydrogenase DNA construct (1487 bp)

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<400> SEQUENCE: 189

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gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgctagcgcc gggccgcctc gactgtatc ttcaagagca tggcaatggt 240
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gaagtggacg tcgcccacca gctttttctc gccgaccggg ttgataccta cgtcgataac 360
taccgcgccc tctttaatca tateccccgg aatcaactcc ggcttcccta cggccgcaat 420
gagaatgtcg gcctgcggcg attcggctgc caggtccctg gtgcgggagt gacagatggt 480
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acagccgtgg ggtgtgcatg gatagaagca tttatcgccg ataaccaggt tgcgcagcgt 660
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gatctgggct gccattgaag taagttagaa gcaggagca ggggaaagaa aattgacaac 1080
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gttttattag cgccagtttg gttttttatc ccattggttg cccaaaatgg gtgaagaagc 1440
tgtcacaagt tggcaacaaa cgatataggt tctctcttct gccgtta 1487

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<210> SEQ ID NO 190
<211> LENGTH: 1565
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 190, example
190: a designer hox-promoter-controlled 10-Methylene-H4 folate
reductase DNA construct (1565 bp)

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<400> SEQUENCE: 190

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
gaggtagata tgctactcta ccttgggacg gggatacagg cgggcttctc tgacgatggt      240
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ctgcagggtg ttaatctggt ccacacagat ggccaccctc tcggccttgg ggtcggaggc      360
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gcaactgggt tgaataaagt cggcgccggc attgatcttc ttctccaggc gcatgaccgc      600
gaactcaaa gggctcgcaa aggggttggc gggcgccgc atgaagaagc ggggttctctg      660
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ggaaagaaaa ttgacaactg tacaagatta atcgcgctctc tgagcaatga ccaatacat     1200
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ttacaagtac aacgcttagt tttattagcg ccagtttggg tttttatccc attggttggc     1500
caaatgggtg gaagaagctg tcacaagttg gcaacaaacg atataggttc tctcttctgc     1560
cgтта                                             1565

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<210> SEQ ID NO 191

<211> LENGTH: 1442

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 191, example
191: a designer hox-promoter-controlled Methyl-H4 folate:
corrinoïd iron-sulfur protein Methyltransferase DNA construct
(1442 bp)

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<400> SEQUENCE: 191

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agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc      60
gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat      120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
gaggtagata tgtaaatccg ctaacaaatc tttacgatat gcctttaaaa agttcttggc      240

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gaaacggtcg ttaccagca gtaaactcgt ggtggtgact gcagtcatga tttttccatc	300
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gttgatgagc ttctcttcg gcagtgtaaa cgagatgttg cttaaaccgg ataactgtctt	420
cactttaaat aacctcttga tttccctcag gcaactggaaa aacatcaccc cgttggtgtg	480
gtttacggcc agggggagaa ccagcggatc gatatacagg ttgctcaaat cataattctt	540
cctgctcagg gtctctatca gtccctcagt tatcttgatc ctttcttcag cgtttttcgg	600
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caatacttet tccagccgct ttttttccat ggatattggag ttgattatca cettgtctct	720
atcgcccttg acggtctcca gacccttttt tattgcctcc cctgctgtgc tatcaatgca	780
taaaggtaca tcagttactt cttgcaactg atgcacaagc cactccatat cggccacttc	840
gtcacagtgg gcggtattta gatccaggta atctgctccc gcttccgect gectgcgggc	900
caggtcctgc acggcccgcg cgtttttgtc attaatgatt tgacggacac tggggatcgc	960
gctatttagt ttttcaccga tgattaacat tgaagtaagt aggaagcagg gagcagggga	1020
aagaaaattg acaactgtac aagattaatc gcgtctctga gcaatgacca aatacatcta	1080
cctccacggt tttcttcag cccctctatc gcgaaagcac aagatattag caagcgtttc	1140
gccaaaattc acatacagct aacaatccct gatctcaatg ctggtgaatt ttctcagtta	1200
acaatcacgc gccaaaattc acaagttgcc gcaattttcc ctgataatc tgaaccaata	1260
acgctgatag gttctagttt aggcggttta actgctgctt atctaggaca gcgatattta	1320
caagtacaac gcttagtttt attagcgcca gtttggtttt ttatcccatt ggttgcccaa	1380
aatgggtgaa gaagctgtca caagttggca acaaacgata taggttctct cttctgccgt	1440
ta	1442

<210> SEQ ID NO 192

<211> LENGTH: 2942

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 192, example
 192: a designer hox-promoter-controlled Corrinoid iron-sulfur
 protein DNA construct (2942 bp)

<400> SEQUENCE: 192

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca	180
gaggtagata tgatggcttt aacaggatta aatatattta aattaacacc aaaaaagaat	240
tgtaaggatt gtggtttccc tacttgctca gctttttcaa tgaaagtagc agcaggagct	300
gtggaaatag gaaaatgtcc tcatatgagt gacgaggcaa tggaaaaatt agctgaagct	360
actgcaccaa ttatgaagac aataactatt ggtaaggag ataataaata taaattaggt	420
ggagaaactg ttttattccg tcatgaaaaa acttttgtaa atagaaatag atttgcagtt	480
gcattttccc atagtatgga tgatgcagaa gtagatgcta agatccaaca tataaaagat	540
gtagattatg ttagaatcgg tgaacaaatg aaaaccgaat ttgctgcaat aaaatatgca	600
ggaaataaag acaaatatct tgctttaata acaaaaataa aagcaagtgg agtaaaagta	660
gcttatgccc tagtttgta agatgtagca gtaataaag aagctcttcc actagttaaa	720

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ttagtagaag aaatacaaaa attaggatataagaacttag tacttgatcc aggtggaaaa	900
tccattaaaag aagcttttga aaatacagtt caaattagaa gaataaatat tgaaggtcag	960
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agtgatatgg attattcaag agcacttcct ctttatagta taagacagaa tgtatttaca	1140
gatccacaaa aaccaatgac agttgatttg ggtatacatg gaattaacaa cccagatgaa	1200
aactcaccag tattatgtac tgttgacttt gctcttactt acttcctagt ttcaggagaa	1260
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caagtaaac gcttagtttt attagcgcca gtttggtttt ttatcccatt ggttgcccaa	2880
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ta	2942

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<210> SEQ ID NO 193
<211> LENGTH: 4859
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 193, example
      193: a designer hox-promoter-controlled CO dehydrogenase/acetyl-
      CoA synthase DNA construct (4859 bp)

<400> SEQUENCE: 193
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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca      180
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gaaatccttt taaaccagcg catccggacc tatgggtcccg accatcccgt cggttatcct      360
gatacagcct attacctgcc ggttattcgc tgtttcagcg ggaagagggt caaaaaactg      420
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gagaatgccc gcctggccgg ggaagccacc tgggatgcgg ccgagatcat tgaagccctg      540
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gctattatcc tgggtcgagc caaagactcg aagccctgg ccaaaatcgt caaggaactc      720
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<210> SEQ ID NO 194

<211> LENGTH: 6428

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 194, example

194: a designer hox-promoter-controlled F420 synthesis enzymes DNA construct (6428 bp)

<400> SEQUENCE: 194

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
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ccaataacgc tgataggttc tagtttaggc ggtttaactg ctgcttatct aggacagcga 6300
tatttacaag tacaacgctt agttttatta gcgccagttt ggttttttat cccattgggt 6360
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tgccgtta 6428

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<210> SEQ ID NO 195

<211> LENGTH: 1778

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 195, example
195: a designer hox-promoter-controlled Pyridoxal phosphate-
dependent L-tyrosine decarboxylase (mfnA for methanofuran
synthesis) DNA construct (1778 bp)

```

<400> SEQUENCE: 195

```

agaaaatctg gcaccacacc gcagaaatat aggggctagg agttgagggt actctggttc 60
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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgttgagggg gcatggggtc gacgaagata cgataatcgg ggagctaaag 240
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ttaatcgcgt ctctgagcaa tgaccaata catctacctc cacggttttc ttccagcccc 1440

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ctatctgcga aagcacaaga tattagcaag cgtttcgccc aaattcacat acagctaaca 1500
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gttgccgcaa ttttccctga taattctgaa ccaataacgc tgataggttc tagtttaggc 1620
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<210> SEQ ID NO 196

<211> LENGTH: 3215

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

```

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 196, example
196: a designer hox-promoter-controlled Methanopterin synthesis
enzymes DNA construct (3215 bp)

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<400> SEQUENCE: 196

```

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agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgatgacagt ttcagaatth ccagatactc aggataaaca accatccatt 240
ccaatatcac taacaagagt tggagtaaca ggtgtaaaga aattaataaa aataaaaaga 300
gaagataaac gtccctattat tctaatacca acatttgatg cttttgtaga tttacctagt 360
actcagaaaag gactacacat gtctagaaat ccagaagcta tatctgaaat tgttgatgag 420
gctgcaaatc aatcggaaat tcatcttgaa aatatatgtg caaatcttgt aaaaagatta 480
cttgaaaaac atgaatatgc attacatgca gaaacagagg caaggggaga atatattata 540
aataaattat ctccagtatc taaaagaaaa acacaggaaa caacacatat catagcaaga 600
gcaattgcta tgaagatga tgagggtaat atttctgtta gaaaaatgat tggtgcaaga 660
gttattggaa tgactgtatg tccatgtgca caagaatctg ttgaaaagga ttctaagat 720
aaattactgg aatthtttaga tgaagaaact acacaaaaag tgttgatgt agtaacattt 780
gcttctcata atcaaggggg tgttgggaca atacttcttg aagtaccaga aaaaacagat 840
gttaatgtgg atgatttaat aaaaataata caggatgcta tgagtctctc tgtttgtgaa 900
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<210> SEQ ID NO 197

<211> LENGTH: 4226

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 197, example
 197: a designer hox-promoter-controlled Coenzyme M synthesis
 enzymes DNA construct (4226 bp)

<400> SEQUENCE: 197

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gtcaagcatt tgggatgatt tcccctcaca agttcctcaa attattctcc tataaacaat 120
agatataagg tcaaaacttg agttatgagt gctgagtaaa aaattactct ccacgcctca 180
gaggtagata tgtaaagat tcacctttcc gagagtatct cccctcaagc ctctttttat 240
ggtttcaagg gctgttatct cttcgggggg tatgtttcca aggttaacgt ctggtcctat 300

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gacacagtgc tgacgctgcc gtgcgataaa ataaagaatt tactggcgat ggtcccctcg 2760
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<210> SEQ ID NO 198

<211> LENGTH: 5198

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct- SEQ ID NO: 198, example
198: a designer hox-promoter-controlled Coenzyme B synthesis
enzymes DNA construct (5198 bp)

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<400> SEQUENCE: 198

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gaggtagata tgttgcccga tcgggtacgg atcttcgaca cgacgctaag agacggtgag 240
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gtgaaggggg	acatagacgc	ggcgatcgac	gccgacgtgg	actgcgtgca	cgtgttcata	480
gccacgtcgg	acatccacct	cagatacaag	ttggagatgt	cccgggaaga	ggcattggag	540
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1. A method for autotrophic production of butanol and related higher alcohols comprising:

introducing a transgenic autotrophic organism into a reactor system, the transgenic autotrophic organism comprising transgenes coding for a set of enzymes configured to confer a hydrogenotrophic pathway for production of a higher alcohol comprising at least four carbon atoms;

using reducing power such as NADPH, reduced ferredoxin, and energy ATP associated with the transgenic autotrophic organism acquired from hydrogenotrophic process in the biological reactor to synthesize the higher alcohol from carbon dioxide and water; and

using a product separation process to harvest the synthesized alcohol from the photobioreactor.

2. The method of claim 1, wherein:

said designer transgenic autotrophic organism comprises at least one of designer Calvin-cycle-channeled pathways and designer hydrogenotrophic pathways for producing at least one of the higher alcohols selected from the group consisting of: 1-butanol, 2-methyl-1-butanol, isobutanol, 3-methyl-1-butanol, 1-hexanol, 1-octanol, 1-pentanol, 1-heptanol, 3-methyl-1-pentanol, 4-methyl-1-hexanol, 5-methyl-1-heptanol, 4-methyl-1-pentanol, 5-methyl-1-hexanol, 6-methyl-1-heptanol and combinations thereof.

3. The method of claim 1, wherein the transgenic autotrophic organism comprises at least one of a transgenic designer plant or transgenic designer plant cell, or bacterial cell selected from the group consisting of blue-green algae (oxyphotobacteria including cyanobacteria and oxychlorobacteria), hydrogenotrophic bacteria, methanogens, aquatic plants, plant cells, green algae, red algae, brown algae, diatoms, marine algae, freshwater algae, salt-tolerant algal strains, cold-tolerant algal strains, heat-tolerant algal strains, antenna-pigment-deficient mutants, butanol-tolerant algal strains, higher-alcohols-tolerant algal strains, butanol-tolerant oxyphotobacteria, butanol-tolerant hydrogenotrophic bacteria and methanogens, higher-alcohols-tolerant oxyphotobacteria and hydrogenotrophic bacteria or methanogens.

4. The method of claim 1, wherein said transgenic autotrophic organism comprises a set of designer genes that express a designer anaerobic hydrogenotrophic butanol-production-pathway system comprising: energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F₄₂₀-reducing hydrogenase (Frh), native (or heterologous) soluble hydrogenase (SH), heterodissulfide reductase (Hdr), formylmethanofuran dehydrogenase, formyl transferase, 10-methenyl-tetrahydromethanopterin cyclohydrolase, 10-methylene-H₄ methanopterin dehydrogenase, 10-methylene-H₄-methanopterin reductase, methyl-H₄-methanopterin: corrinoid iron-sulfur protein methyltransferase, corrinoid

iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase.

5. The method of claim 1, wherein the transgenic autotrophic organism comprises bacteria selected from the group consisting of *Thermosynechococcus elongatus* BP-1, *Nostoc* sp. PCC 7120, *Synechococcus elongatus* PCC 6301, *Synechococcus* sp. strain PCC 7942, *Synechococcus* sp. strain PCC 7002, *Synechocystis* sp. strain PCC 6803, *Prochlorococcus marinus* MED4, *Prochlorococcus marinus* MIT 9313, *Prochlorococcus marinus* NATL1A, *Prochlorococcus* SS120, *Spirulina platensis* (*Arthrospira platensis*), *Spirulina pacifica*, *Lyngbya majuscula*, *Anabaena* sp., *Synechocystis* sp., *Synechococcus elongatus*, *Synechococcus* (MC-A), *Trichodesmium* sp., *Richelia intracellularis*, *Synechococcus* WH7803, *Synechococcus* WH8102, *Nostoc punctiforme*, *Synechococcus* sp. strain PCC 7943, *Synechocystis* PCC 6714 phycocyanin-deficient mutant PD-1, *Cyanothece* strain 51142, *Cyanothece* sp. CCY0110, *Oscillatoria limosa*, *Lyngbya majuscula*, *Symploca muscorum*, *Gloeobacter violaceus*, *Prochloron didemni*, *Prochlorothrix hollandica*, *Synechococcus* (MC-A), *Trichodesmium* sp., *Richelia intracellularis*, *Prochlorococcus marinus*, *Prochlorococcus* SS120, *Synechococcus* WH8102, *Lyngbya majuscula*, *Symploca muscorum*, *Synechococcus bigranulatus*, *cryophilic Oscillatoria* sp., *Phormidium* sp., *Nostoc* sp.-1, *Calothrix parietina*, thermophilic *Synechococcus bigranulatus*, *Synechococcus lividus*, thermophilic *Mastigocladus laminosus*, *Chlorogloeopsis fritschii* PCC 6912, *Synechococcus vulcanus*, *Synechococcus* sp. strain MA4, *Synechococcus* sp. strain MA19, *Methanocella paludicola* SANAE, *Acinetobacter baumannii* ABNIH3, *Acinetobacter baumannii* ABNIH4, *Acinetobacter* sp. DR1, *Agrobacterium* sp. H13-3; *Agrobacterium vitis* S4, *Alcaligenes* sp., *Allochrochromatium vinosum* DSM 180, *Amycolatopsis mediterranei* S699, *Anoxybacillus flavithermus* WK1, *Aquifex aeolicus* VF5, *Archaeoglobus fulgidus* DSM 4304, *Archaeoglobus veneficus* SNP6, *Azospirillum* sp. B510, *Burkholderia cenocepacia* HI2424, *Caldicellulosiruptor bescii* DSM 6725, *Carboxydotherrmus hydrogenoformans*, *Centipeda periodontii* DSM 2778, *Clostridium autoethanogenum*, *Clostridium ragsdalei*, *Clostridium sticklandii* DSM 519, *Clostridium sticklandii*, *Corynebacterium glutamicum*, *Cupriavidus metallidurans* CH34, *Cupriavidus necator* N-1, *Desulfobacca acetoxidans* DSM 11109, *Exiguobacterium* sp. AT1b, *Ferrimonas balearica* DSM 9799, *Ferroglobus placidus* DSM 10642, *Geobacillus kaustophilus* HTA426, *Helicobacter bilis* ATCC 43879, *Herbaspirillum seropedicae* SmR1, *Hydrogenobacter thermophilus* TK-6, *Hydrogenovibrio marinus*, *Klebsiella variicola* At-22, *Methanobacterium* sp. SWAN-1, *Methanobrevibacter ruminantium* M1, *Methanocaldococcus*

fervens AG86, *Methanocaldococcus infernus* ME, *Methanocaldococcus jannaschii*, *Methanocaldococcus* sp. FS406-22, *Methanocaldococcus vulcanius* M7, *Methanococcus aeolicus* Nankai-3, *Methanococcus maripaludis* C6, *Methanococcus maripaludis* S2, *Methanococcus voltae* A3, *Methanocorpusculum labreanum* Z, *Methanoculleus marisnigri* JR1, *Methanohalophilus mahii* DSM 5219, *Methanolinea tarda* NOBI-1, *Methanoplanus petrolearius* DSM 11571, *Methanoplanus petrolearius*, *Methanopyrus kandleri* AV19, *Methanoregula boonei* 6A8, *Methanosaeta harundinacea* 6Ac, *Methanosalsum zhilinae* DSM 4017, *Methanosarcina acetivorans* C2A, *Methanosarcina barkeri* str. *Fusaro*, *Methanosarcina mazei* Go1, *Methanosphaera stadtmanae*, *Methanospirillum hungatei* JF-1, *Methanothermobacter marburgensis* str. *Marburg*, *Methanothermobacter marburgensis*, *Methanothermobacter thermoautotrophicus*, *Methanothermococcus okinawensis* IH1, *Methanothermus fervidus* DSM 2088, *Methylobacillus flagellates*, *Methylobacterium organophilum*, *Methylococcus capsulatus*, *Methylomicrobium kenyense*, *Methylomonas methanica* MC09, *Methylomonas* sp. LW13, *Methylosinus* sp. LW2, *Methylosinus trichosporium* OB3b, *Methylotenera mobilis* JLW8, *Methylotenera versatilis* 301, *Methylovorus glucosetrophus* SIP3-4, *Moorella thermoacetica* ATCC 39073, *Moorella thermoacetica*, *Oligotropha carboxidovorans* OM5, *Paenibacillus terse* HPL-003, *Pelotomaculum thermopropionicum* SI, *Planctomyces brasiliensis* DSM 5305, *Pyrococcus furiosus* DSM 3638, *Pyrococcus horikoshii* OT3, *Pyrococcus yayanosii* CH1, *Ralstonia eutropha* H16, *Rubrivivax* sp., *Selenomonas noxia* ATCC 43541, *Shewanella baltica* BA175, *Stenotrophomonas* sp. SKA14, *Synechococcus* sp. JA-2-3B' a(2-13), *Synechococcus* sp. JA-3-3Ab, *Thermococcus gammatolerans* EJ3, *Thermococcus kodakarensis* KOD1, *Thermococcus onnurineus* NA1, *Thermococcus* sp. 4557, *Thermodesulfator indicus* DSM 15286, *Thermosifilum pendens* Hrk 5, *Thermotoga lettingae* TMO, *Thermotoga petrophila* RKU-1, *Thiocapsa roseopersicina*, *Thiomonas intermedia* K12, *Xanthobacter autotrophicus*, *Yersinia pestis Antigua*, and *Thermosynechococcus elongatus*.

6. The method of claim 1, wherein the transgenic autotrophic organism comprises a biosafety-guarded feature selected from the group consisting of a designer proton-channel gene inducible under pre-determined inducing conditions, a designer cell-division-cycle iRNA gene inducible under pre-determined inducing conditions, a high-CO₂-requiring mutant as a host organism for transformation with designer biofuel-production-pathway genes in creating designer cell-division-controllable autotrophic organisms, and combinations thereof; and wherein said transgenic autotrophic organism comprises a set of designer genes exemplified with exemplary designer DNA constructs of SEQ ID NOS. 1-198 shown in the sequence listings for expressing at least one of the enzymes selected from the group consisting of oxygen-tolerant soluble hydrogenase (SH), oxygen-tolerant membrane bound hydrogenase (MBH), energy converting hydrogenase (Ech), methyl-H4MPT: coenzyme-M methyltransferase (Mtr), methyl-coenzyme M reductase (Mcr), heterodisulfide reductase (Hdr), [NiFe]-hydrogenase (Mvh), Coenzyme F₄₂₀-reducing hydrogenase (Frh), A₁A_o-ATP synthase, formate dehydrogenase, 10-formyl-H₄ folate synthetase, methenyltetrahydrofolate cyclohydrolase, 10-methylene-H₄ folate dehydrogenase, 10-methylene-H₄ folate reductase, methyl-H₄ folate: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-

sulfur protein, CO dehydrogenase/acetyl-CoA synthase, formylmethanofuran dehydrogenase, formyl transferase, 10-methenyl-tetrahydromethanopterin cyclohydrolase, 10-methylene-H₄ methanopterin dehydrogenase, 10-methylene-H₄-methanopterin reductase, methyl-H₄-methanopterin: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, butanol dehydrogenase, 2-keto acid decarboxylase, alcohol dehydrogenase, 2-methylbutyraldehyde reductase, 3-methylbutanal reductase, hexanol dehydrogenase, octanol dehydrogenase, and short-chain alcohol dehydrogenase.

7. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, 2-isopropylmalate synthase, isopropylmalate isomerase, 2-keto acid decarboxylase, alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and butanol dehydrogenase.

8. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer anaerobic hydrogenotrophic system and butanol-production pathway selected from the group consisting of energy converting hydrogenase (Ech), [NiFe]-hydrogenase Mvh, Coenzyme F₄₂₀-reducing hydrogenase (Frh), soluble hydrogenase (SH), reduced ferredoxin (Fd_{red}²⁻), and heterodisulfide reductase (Hdr), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, 2-isopropylmalate synthase, isopropylmalate isomerase, 3-isopropylmalate dehydrogenase, 2-keto acid decarboxylase, and NAD-dependent alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, butanol dehydrogenase and combinations thereof.

9. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer hydrogenotrophic methanogenic 2-methylbutanol-production pathway selected from the group consisting of methyl-H4MPT: coenzyme-M methyltransferase Mtr, A₁A_o-ATP synthase, methyl-coenzyme M reductase Mcr, energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F₄₂₀-reducing hydrogenase (Frh), soluble hydrogenase (SH), heterodisulfide reductase (Hdr), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, NAD-dependent alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and 2-methylbutyraldehyde reductase.

10. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of membrane bound hydrogenase (MBH), soluble

hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, and NAD dependent alcohol dehydrogenase, NADPH dependent alcohol dehydrogenase, and 2-methylbutyraldehyde reductase.

11. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of methyl-H4MPT: coenzyme-M methyltransferase Mtr, A_1A_0 -ATP synthase, energy converting hydrogenase (Ech), [NiFe]-hydrogenase Mvh, Coenzyme F_{420} -reducing hydrogenase (Frh), native (or heterologous) soluble hydrogenase (SH), reduced ferredoxin (Fd_{red}^{2-}), methyl-coenzyme M reductase Mcr, heterodisulfide reductase (Hdr), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-keto acid decarboxylase, and NAD-dependent alcohol dehydrogenase, and NADPH-dependent alcohol dehydrogenase.

12. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, 2-isopropylmalate synthase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, 2-keto acid decarboxylase, and NAD-dependent alcohol dehydrogenase, NADPH-dependent alcohol dehydrogenase, and 3-methylbutanal reductase.

13. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer anaerobic reductive-acetyl-CoA butanol-production pathway selected from the group consisting of: formate dehydrogenase, 10-formyl- H_4 folate synthetase, methenyltetrahydrofolate cyclohydrolase, 10-methylene- H_4 folate dehydrogenase, 10-methylene- H_4 folate reductase, methyl- H_4 folate: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, butanol dehydrogenase, and alcohol dehydrogenase.

14. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, 2-isopropylmalate synthase, isopropylmalate isomerase, 3-isopropylmalate dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, short-chain alcohol dehydrogenase, hexanol dehydrogenase, designer isopropyl-

malate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, and designer short-chain alcohol dehydrogenase.

15. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, 2-isopropylmalate synthase, isopropylmalate isomerase, 3-isopropylmalate dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, short-chain alcohol dehydrogenase, hexanol dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, and designer short-chain alcohol dehydrogenase.

16. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer hydrogenotrophic Calvin-cycle-channeled pathway selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, citramalate synthase, 2-methylmalate dehydratase, 3-isopropylmalate dehydratase, 3-isopropylmalate dehydrogenase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, short-chain alcohol dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, and designer short-chain alcohol dehydrogenase.

17. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer hydrogenotrophic Calvin-cycle-channeled pathway selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, aspartate aminotransferase, aspartokinase, aspartate-semialdehyde dehydrogenase, homoserine dehydrogenase, homoserine kinase, threonine synthase, threonine ammonia-lyase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, short-chain alcohol dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, and designer short-chain alcohol dehydrogenase.

18. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer

hydrogenotrophic Calvin-cycle-channeled pathway selected from the group consisting of membrane bound hydrogenase (MBH), soluble hydrogenase (SH), NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, enolase, pyruvate kinase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxy-acid dehydratase, isopropylmalate synthase, dehydratase, 3-isopropylmalate dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, short-chain alcohol dehydrogenase, designer isopropylmalate synthase, designer isopropylmalate isomerase, designer 3-isopropylmalate dehydrogenase, designer 2-keto acid decarboxylase, and designer short-chain alcohol dehydrogenase.

19. The method of claim 1, wherein the set of enzymes comprises at least one of the enzymes conferring a designer methanogenic hydrogenotrophic butanol-production-pathway selected from the group consisting of: methyl-H4MPT: coenzyme-M methyltransferase Mtr, A_1A_o -ATP synthase, methyl-coenzyme M reductase Mcr, energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F_{420} -reducing hydrogenase (Frh), soluble hydrogenase (SH), heterodissulfide reductase (Hdr), formate dehydrogenase, 10-formyl- H_4 folate synthetase, methenyltetrahydrofolate cyclohydrolase, 10-methylene- H_4 folate dehydrogenase, 10-methylene- H_4 folate reductase, methyl- H_4 folate: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, butanol dehydrogenase, and alcohol dehydrogenase.

20. The method of claim 1, wherein the designer transgenic organism a designer autotrophic organism comprises a set of

designer genes that express a designer methanogenic hydrogenotrophic butanol-production-pathway system comprising: methyl-H4MPT: coenzyme-M methyltransferase Mtr, A_1A_o -ATP synthase, methyl-coenzyme M reductase Mcr, energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F_{420} -reducing hydrogenase (Frh), soluble hydrogenase (SH), heterodissulfide reductase (Hdr), formate dehydrogenase, 10-formyl- H_4 folate synthetase, methenyltetrahydrofolate cyclohydrolase, 10-methylene- H_4 folate dehydrogenase, 10-methylene- H_4 folate reductase, methyl- H_4 folate: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase; and wherein said autotrophic organism comprise a set of designer genes that express a designer methanogenic hydrogenotrophic butanol-production-pathway system comprising: methyl-H4MPT: coenzyme-M methyltransferase Mtr, native (or heterologous) A_1A_o -ATP synthase, methyl-coenzyme M reductase Mcr, energy converting hydrogenase (Ech), [NiFe]-hydrogenase (Mvh), Coenzyme F_{420} -reducing hydrogenase (Frh), native (or heterologous) soluble hydrogenase (SH), heterodissulfide reductase (Hdr), formylmethanofuran dehydrogenase, formyl transferase, 10-methenyl-tetrahydromethanopterin cyclohydrolase, 10-methylene- H_4 methanopterin dehydrogenase, 10-methylene- H_4 -methanopterin reductase, methyl- H_4 -methanopterin: corrinoid iron-sulfur protein methyltransferase, corrinoid iron-sulfur protein, CO dehydrogenase/acetyl-CoA synthase, thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase, and butanol dehydrogenase.

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